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Gamma Radiation and Tissue Culture Induced Variability in Moricandia (*Moricandia sinaica* Boiss) with Genetic Assessment by ISSR markers and Phytochemical Profiling of Organosulfur Compounds via GC–MS

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ABSTRACT

Moricandia sinaica is a member of genus Moricandia. *Moricandia sinaica* is used in traditional medicine to alleviate certain disorders such as syphilis and exhibits analgesic, anti-inflammatory, antipyretic, antioxidant, and antigenotoxic properties. Buds were cultured on Murashige and Skoog (MS) solid medium with different strength with and without hormones asBAP and Kin. The best medium was half strength MS without hormones. Plantlets were exposed to different gamma radiation doses 10, 20, 30, 40, 50 and 60 Gy.The morphological parameters of irradiated plantlets were decreased by increasing gamma radiation doses. The lethal dose was 40 Gy and the optimal dose for explant survival was 10 Gy. Genetic diversity was evaluated by ISSR markers using 20 primers. 149 bands were the total amplified bands by ISSR markers. The mean of polymorphism was 58.55%.The polymorphic information content (PIC) with an average 0.363 (PIC<0.5) classifying most primers as moderately informative. High PIC values (0.371–0.374)and discriminating power confirmed substantial genomic variation induced by Gamma radiation treatments. The effect of gamma radiation on organosulfur compounds as analyzed by GC- MS was limited to increased production as2-Myristynoyl pantetheine; reduced production as Ethanimidothioic acid,2-(Dimethylamino)N[(Methylamino)Carbonyl]Oxy]-2-Oxo-,Methyl Ester; disappearance of some compounds as Thiocyanic Acid, Octyl Ester and 2-[3-Cyclohexylaminopropylamino] ethylthiophosphate and formation of some new compounds as Leukotriene D4 methyl ester, Cefazolin, Meraptal, Pentaacetate, Mannitol, tert-Hexadecanethiol, 2-Ethyl-5,7-Dimethyl.

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Introduction

Moricandia sinaica (*Moricandia sinaica* Boiss) belongs to Brassicaceae family (Cruciferae). *Moricandia sinaica* is an annual to perennial plant, were evaluated analgesic, syphilis, pain, fever, anti-inflammatory and antipyretic activities. M. sinaica exhibited remarkable cardiac and nephroprotective efects **(El-mekkawy *et al*., 2020; Riaz Ullah *et al*., 2023 and El-Sayed *et al*., 2024**). Moricandia is the solitary genus consists of eight weedy species among which five species i.e., *M. arvensis, M. nitens, M. sinaica, M. spinosa and M. suffruticosa* are with C3-C4 intermediated photosynthetic mechanism. Moreover, it is used as donors' genes responsible for their tolerance to the antagonistic ecological conditions and the production of active ingredientsare of vital for genetic engineering to crop species through techniques as protoplast fusion **(Zhihong and Jinling, 1999).** The genus Moricandia allocating in the southern Mediterranean and North African regions. Egyptian desert contains many natural economic and medicinal plants like *Moricandia sinaica*, which may be important for the present and future generations **(Soliman *et al.,* 2012)**. Tissue culture technique offers better control than *in vivo* growth conditions which illustrated the responses of plants to unsuitable environmental conditions as biotic and abiotic stress, rapid multiplication of rare species and genetic transformation of plants. Moreover, it's a rapid way for producing high quality genetically uniform plants and preserve the genetic gain. In addition, Micropropagation technique helped the identification and isolation of bioactive phytocompounds has numerous industrial applications. production of plant derived bioactive compounds It provides potential benefits for different industries which include food, pharmaceutical and cosmetics, (**Espinosa-Leal *et al*., 2018**; **Sobieh *et al*., 2018; Rout *et al*., 2000; Rout *et al*., 2006 and Wijerathna-Yapa and Hiti-Bandaralage, 2023).** Gamma radiation estimated with ^{60}Co has high penetration potential, carriages no risk to the environment in addition may be used to irradiate cells, tissues, organs and whole plants **(Spinoso-Castillo *et al*., 2021).**Gamma radiation damages plants directly by transfer their high energy to DNA molecules to ionizing and damaging DNA and cellular components, otherwise indirectly by causing water radiolysis to produce free radicals and reactive oxygen species (ROS). In cooperation direct and indirect damage contribute to DNA damage, genomic instability, proteins, and lipids within the plant cell. This mutilation is influenced by factors such as total dose and dose rate of gamma radiation and the specific plant species, which can affect their ability to cope with and repair the damage. So, the milder exposure affects plant morphology, physiology, secondary metabolites and cell abnormalities. While, in severe cases of acute gamma radiation doses, the cumulative damage can be lethal to the plant. (**Kovács and Keresztes, 2002; El-Fiki *et al*., 2018; Choi *et al*., 2021; Awad *et al*., 2022 and Saloua and Alansari, 2025).** Moreover, Different radiation doses can lead to different types and levels of genetic variation, which can be visualized by Inter simple sequence repeat (ISSR) marker. ISSR marker is based on polymerase chain reaction **(Zebarjadi, *et al*., 2016).** The ISSR marker established to be a fast, simple, steady, and highly informative method for DNA fingerprinting. This technique relies on amplifying a segment of DNA that is at a distance that can be found between two identical microsatellite repeat regions oriented in opposite directions. This technique is valuable for plant breeding to induce and then monitor genetic diversity for crop improvement and distinguishing irradiated lines from the control based on their molecular fingerprinting **(Wang *et al*., 2020 and El -Gazzar *et al*., 2023**). Gamma radiation causes molecular level changes in the plant cells, this directed to synthesis wide range of secondary metabolites which serve as an abundant source of phyotochemicals and bioactive compounds **(Nielsen *et al*., 2019).** The aerial parts extract of M. sinaica exerts potential analgesic, anti-inflammatory and antipyretic effects. Organo-Sulfur compounds, often referred to as S-containing compounds, that characterized by the presence of sulfur atoms in their molecular structures and are a variety of secondary metabolites in plants. Moreover, these compounds play a chief role in plant defense, growth regulation, and ecological interactions which confer unique chemical properties and biological activities **(Castro *et al*., 2023).** Plants are the primary fabricators of organic sulfur compounds. sulfur assimilation is chloroplast-associated where photosynthetic reductant and ATP reduce sulfur compounds (such as sulfate) for biosynthesis of sulfur containing amino acids like cysteine, methionine. Cysteine serves also for the synthesis of glutathione and provides sulfur to many other molecules including protein cofactors or vitamins and antioxidants. Moreover, Cysteine is at the center of plant metabolism and plays an important role in plant life processes, especially stress resistance **(De Bont *et al*., 2022; Lv *et al*., 2025)**. The recent study usage gamma irradiation as a physical mutagen to induce genetic variations with *Moricandia sinaica in vitro*. Perusing variations morphologically and molecular through ISSR marker. Furthermore, the alterations in organosulfur compounds using GC- MS.

Materials and Methods

Plant materials

Moricandia sinaica plants were obtained from South Western Sinai fromBotany and Microbiology Department, Faculty of Science (Boy's Branch), Al-Azhar University, Cairo, Egypt)**.** Surface sterilization of siliques was carried out by washing in soap for 2 min, then washed under tap water for 15 min. Subsequently, dipping in Clorox (30% containing

5% sodium hypochlorite) for 15 minwith Shake vigorously, followed by three rinses in sterile distilled water. *Moricandia sinaica* plantlets were propagated *in vitro* by seeds in siliques cultured on (3/4 strength MS medium supplemented with 0.2 mg/lBAP + 0.1 mg/lKIN+30 g/l sugar+ 6 g/l agar at pH 5.8 and autoclaving at 1.5bar and121°C for 18 minutes). The cultures jars were incubated in a growth chamber at 25 ± 2°c under photoperiod 16h of 3000 lux intensity**as Fig. (1).** Also, used different strengths of MS medium **(Murashige and Skoog, 1962)** as (full strengths, 3/4 and 1/2 strength MS medium), also study the influence of addition growth regulator as0.2mg/l BAP + 0.2mg/l KIN to different strengths MS medium (full strengths, 3/4 and 1/2 strength MS medium). Added 30 g/l sugar+ 6 g/l agar at pH 5.8 and autoclaving at 1.5bar and 121°C for 18 minutes to all media. All media were studied to choose the best medium to propagate *Moricandia sinaica* plantlets. After choose the best media and propagated, the plantlets were exposed to different doses of gamma radiation.

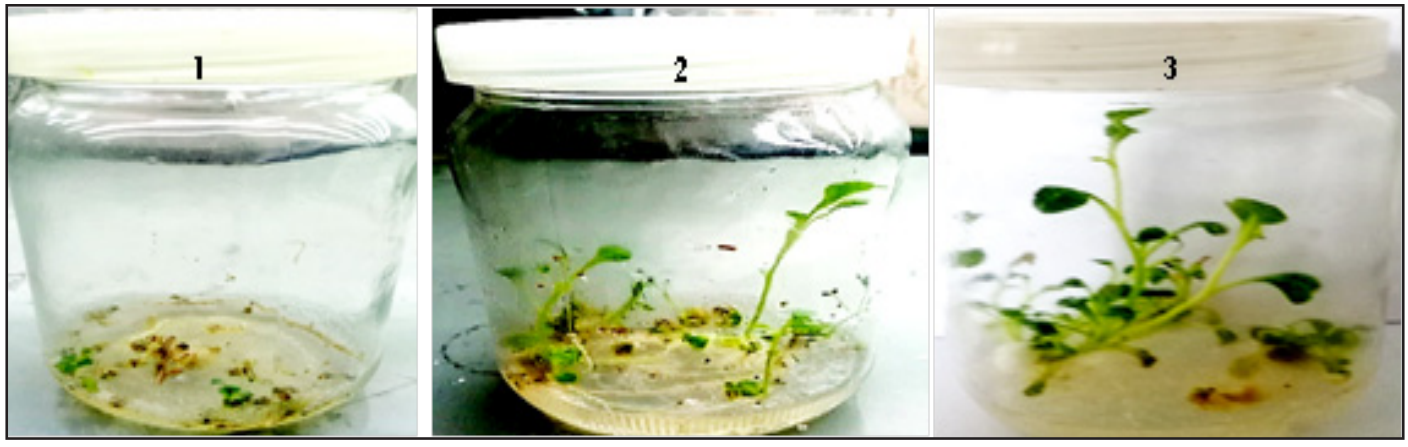

Figure (1): Steps of Moricandia sinaica micropropagation

Gamma Irradiation Treatments

Gamma irradiation was carried out with ^{60}Co source at the dose rate 0.709 KGy /h at National Centre for Radiation Research and Technology, Cairo, Egypt. Moricandia plantlets were exposed to gamma radiation doses of 10, 20, 30, 40,50 and 60 Gy. Then buds were cultivated on culture medium (1/2 MS medium + 6 g/l agar + 3% sucrose at pH 5.8 and autoclaving at 1.5bar and 121°C for 18 minutes).

Genomic DNA Extraction

Total genomic DNA was isolated from seven treatments of plantlets (Control,10, 20, 30, 40, 50 and 60Gy). The plantlets samples, were weighing approximately 0.5g then were ground into a fine powder using liquid nitrogen according to **Anderson *et al.* (1992)**, to enhance the DNA's quality: two consecutive extractions were performed using a mixture of phenol and chloroform (1:1), followed by an additional wash with 97% ethanol (kept at −20 °C for one hour) and 70% pre-cooled ethanol, respectively. The yield and quality of the DNA were evaluated using a

spectrophotometer and gel electrophoresis. For the analysis of genetic markers, 20 ISSR primers.

ISSR – PCR amplification

20 different ISSR primers are G-02, G-03, CCA, CGA, GT, AG, CT, CA, ACA, ISSR2, ISSR7, ISSR10, ISSR11, ISSR12, ISSR13, ISSR14, ISSR16, ISSR18, ISSR24 and G-01 which have 11, 14, 15, 16, 17, 18 and 19 nucleotides based on di- or tri- nucleotide ISSR repeats as **Table (1).** Amplification reactions were performed in a 25 µl volume, containing: 12.5 µl 2×EasyTaq® PCR SuperMix (Lot#Q20527 ,1.25ml), 1 µl primer, 1µlof genomic DNA and 9.5 µl sterile distilled water. The thermocycling program utilized was as follows: an initial cycle at 94 °C for2 min.Then amplified for 40 cycles consisting of the 30s at 94°C, 30s at 41.5°C and 2 min. at 72 °C followed by 5 min. incubation at 72 °C. Amplification products were separated by gel electrophoresis on 0.8% agarose and visualized under UV illumination after staining with ethidium bromide and photographed.

Data analysis

The sizes of ISSR fragments were estimated by comparing the induced bands with the BERUS (100bp) DNA Ladder (WF-WF10407001). ISSR fingerprints were recorded in the binary form (1 = presence of a band and 0 = absence of a band). The percentage of polymorphic fragments in Moricandia were used to evaluate the genetic diversity between control and irradiated treatments. Fragment sizes of ISSR were determined with EgyGene GelAnalyzer4 software **(Ahmed, 2021).** Comparison with the DNA marker. Amplified products were scored as present (1) or absent (0) to form a binary matrix. In order to measure the informativeness of the markers to differentiate between treatments, Total amplified band (TAB), Polymorphic band no. (PBN), % polymorphic band (%PB), Band size/bp (BS/bp), Marker index (*MI*), Polymorphism information content

(PIC), Heterozygosity index (H), Effective multiplex ratio (E), Arithmatic mean of H (H.av), Discriminating power (D), Resolving power (R). were calculated by the usage of iMEC: Online Marker Efficiency Calculator, (https://irscope.shinyapps.io/iMEC/, **Amiryousefi *et al.*, 2018).**

Table 1. Amplification results generated by ISSR primers in Moricandia sinaica plantlets.

No.	Primers	Sequence (5' to 3')	GC%
1	G-02	AGAGGTGGGCAGGTGG	69
2	G-03	GAGGGTGGAGGATCT	60
3	CCA	DDB(CCA)5	56
4	CGA	DHB(CGA)5	56
5	GT	VHV(GT)5G	43
6	AG	HBH(AG)7A	39
7	CT	DYD(CT)7C	44
8	CA	DBDA(CA)7	39
9	ACA	BDB(ACA)5	28
10	ISSR 2	(GA)9C	53
11	ISSR 7	(GA)8C	53
12	ISSR 10	(AG)8G	53
13	ISSR 11	(GA)8G	53
14	ISSR 12	(GA)8A	47
15	ISSR 13	(TC)8C	53
16	ISSR 14	(TC)8G	53
17	ISSR 16	(TG)8A	47
18	ISSR 18	(ATC)6T	32
19	ISSR 24	CACCACCACGC	73
20	G-01	AGGGCTGGAGGAGGGC	75

Extraction of organosulphur compounds

Dried 2grams *Moricandia sinaica* plantlets of treatments (0, 10, 20, 30 and 40Gy) in the shade were grounded to powder. The crude extraction of dried parts of *Moricandia sinaica* was obtained by transferring the powder to 10 ml clean tubes and added 5 ml methanol (Sigma-Aldrich, for HPLC ≥ 99.9%) overnight.

GC–MS analysis

The chemical composition samples of treatments (0, 10, 20, 30 and 40 Gy) performed using GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 60°C and then increased by 5°C /min to 250°C withhold 2 min then increased to 300 with 30 C/min. The injector temperature was kept at 270°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C respectively. The components were identified by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral database **(Abd El-Kareem *et al.*, 2016).**

Statistical Analysis

The data were statistically analyzed using ANOVA analysis to determine the level of significant differences between means as compared to the control at P≤ 0.05 level of significance. The statistical software Costat (http://www.cohort.com/costat.html) was used for all analyses.

Results and Discussion

In Vitro Culture

Tissue culture technique is used in a large scale for economically programs to produce a healthy plant, and used in the production of secondary metabolites and phytoconstituents. Plant tissue culture used for a wide range with various applications in research and industry. In the present study detected the effects of different strengths (full, 3/4 and1/2) of MS media and influence of addition growth regulator as 0.2mg/l BAP + 0.2mg/l KIN on *in vitro* propagation of *Moricandia sinaica*. Seedlings derived from seeds in siliques germinated directly on (3/4 strength MS medium supplemented with 0.2 mg/lBAP + 0.1 mg/lKIN. Then, *Moricandia sinaica* plantlets propagated in vitro on different strength MS medium. Data in **Table (2) and Fig. (2)** were Demonstrated the effect of addition 0.2 mg/l of BAP and 0.2 mg/l of KIN to different strength MS medium.The medium **No. (3)** (1/2 MS medium without hormones) was the best media compared to another media.Medium no. 3 had positively effect on the growth of buds, with bud survival of 96.66% and the mean shoot length of 2.1 cm. The lowest growth was recorded on half strength MS medium **No. (4)** supplemented with 0.2 mg/l of BAP and 0.2 mg/l of KIN. Itrecorded the lowest bud survival percentage 70% and mean shoot length of 1.4 cm. Therefore, the propagation of *Moricandia sinaica* is possible *in vitro* by planlets on ½ strength MS medium.

The need for medicinal plants is increasing therefore; it is necessary to find other ways to produce the amount of plant material that is needed. Propagation through tissue culture offers a

viable alternative for normal propagation because it can also be used as a complimentary strategy for conservation and utilization of genetic resources. Further, in vitro plant regeneration through

vegetative parts is an easy and economical way for obtaining a large number of consistently uniform and truetotype plants within a short span of time **(Abdalla *et al.*, 2022**). This study clear that the ½ MS medium (half strength MS medium without hormones) could be the beneficial effects with optimum growth for *Moricandia sinaica* plantlets. Similar results were obtained by **Ray *et al.* (2006)** who found that the ½ MS basal medium was the best elongation shoots of *Cordyline terminalis* (L.) and **Stamenković *et al.*, (2012)** described that the maximum multiplication rate was completed with ½ MS medium on Campanula velebitica Borbás. Furthermore, depending on plant species, a diluted tissue culture medium could be used with optimal growth (**Noor El-Deen and Abdul-Moneem, 2025**). In addition, media composition is one of the furthermost critical features inducing plant growth (**Rezali *et al.*, 2017; Tütüncü *et al.*, 2019**). Thus, reducing the strength of MS media could increase total chlorophyll (chlorophyll a and chlorophyll b concentration), improving germination rates, promoting more vigorous root and shoot growth, and preventing nutrient toxicity Since MS media contains all the macronutrients, micronutrients and vitamin, changing the strength or concentration of MS media may alter the nutrients component. Therefore, the effect of different medium strength could also affect the plant organogenesis in vitro. The amount of nutrients in culture media for successful culture may vary for different plant species and genotype. A study by **Mukherjee *et al.* (2010)** proved that shoot proliferation of grape rootstock was increased when cultured on MS with half

of nitrate medium. Other than that, **Fakhrul *et al.* (2014)** stated that macronutrients manipulation in MS media could affect the growth of Stevia rebaudiana.

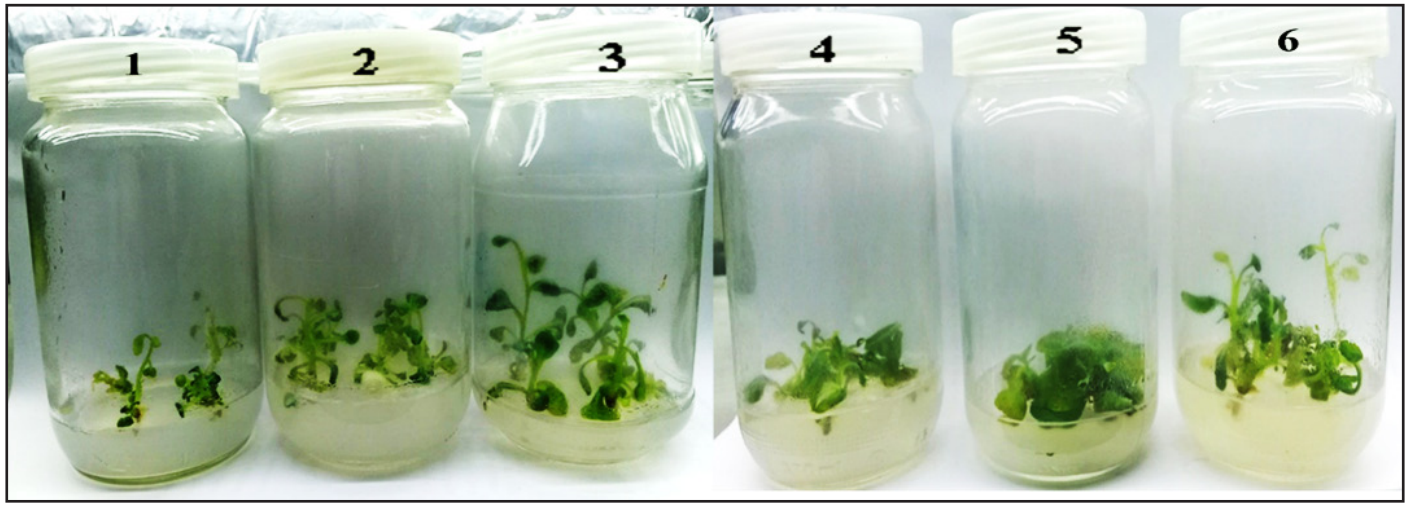

Figure (2): Responses of *Moricandia sinaica* explants to different culture media

Table 2. Responses of Moricandia sinaica explants to different culture media (30buds/media).

No.	Culture media	Complete plantlets	Callus formation	Root formation	No. of growing plantlets	Bud survival percentage %	Sum of shoot length/cm	Mean of shoot length/cm	Mean of shoot No.
1	1xMS	+	-	++	22	73.33	50.5	1.68	2.21
2	3/4xMS	+	-	+++	24	80	53	1.76	2.62
3	1/2xMS	+	-	+++	29	96.66	60.5	2.01	3.4
4	1/2xMS+ $0.2^{mg/l}$ BAP+ $0.2^{mg/l}$ Kin	+	-	+	21	70	42	1.4	1.4
5	3/4xMS+ $0.2^{mg/l}$ BAP+ $0.2^{mg/l}$ Kin	+	-	+	23	76.66	45	1.5	1.5
6	1xMS+ $0.2^{mg/l}$ BAP+ $0.2^{mg/l}$ Kin	+	-	-	25	83.33	50	1.66	1.5

• *+ = Formation of roots* *- = Deformation of roots* *- = Deformation of Callus*

Effect of gamma radiation on some morphological parametres

Effect of Gamma Irradiation on in vitro growth ionizing radiation successfully used for medicinal and aromatic plants, which are important sources of plant secondary metabolites. Determination of optimum dose, radio sensitivity and treatment conditions are most vital for genetic manipulation through the induction of mutation**.** In this investigation, *Moricandia sinaica* plantlets were irradiated with gamma irradiation doses of 10, 20, 30, 40, 50 and 60 Gy). The results as in **Table (3) and Fig (3)** cited that low doses of gamma radiation caused significantly decreasing in survival percentage and another morphological parameter by increase doses. Furthermore, the mortality of explants was increased by increase doses. The dose of 10 Gy caused decreasing in bud survival percentage to about 12% and decreased mean shoot length to 0.81 cm. The usage of dose 20 Gy was induced mortality in bud culture of about 16%. Also, the mean shoot length was affected negatively with the same dose to 1.14 cm. Furthermore, the mortality was increased with the dose of 30, 40 and 50 Gy to 36, 64 and 76% respectively, similarly the mean shoot length decreased to 1.03, 1.23 and 1.70cm. The severe depression in survival percentage to 84%, and the shoot length was decreased to 2.08 cm with the dose of 60 Gy. These results are agreement with **Awad *et al.* (2022)** who stated the presence of differences in morphological and growth parameters between un-irradiated and irradiated treatments. The growth of plants irradiated with a high dose of gamma rays (50 Gy) was significantly inhibited.

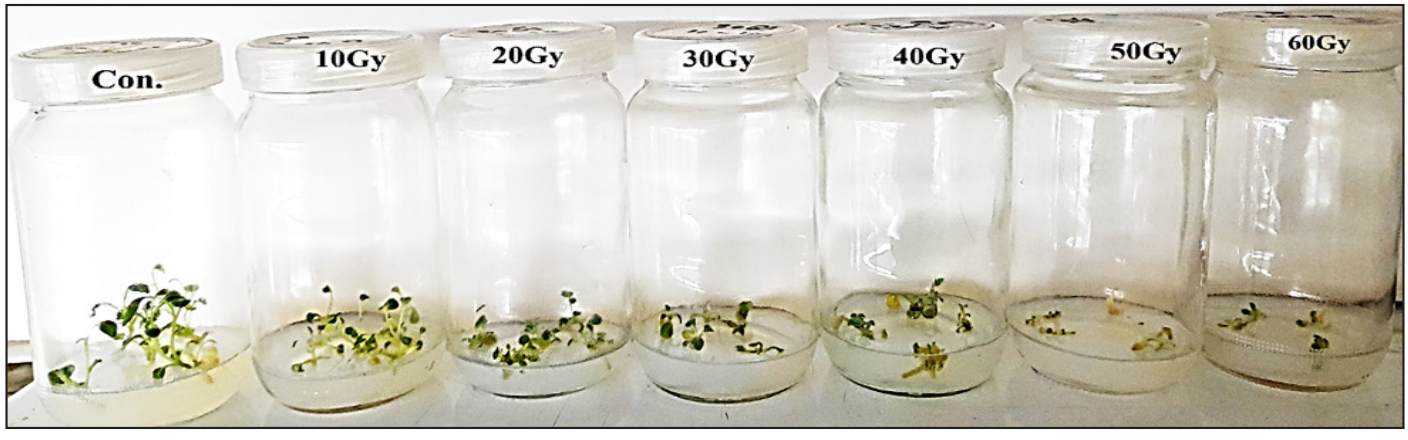

Figure (3): ResEffect of gamma radiation doses in *Moricandia sinaica* plantlets

The reasons of inhibition plant growth and development were by direct and indirect effects of gamma radiation.

Also, photosynthetic capacity was reduced by deformation of the thylakoid structure in chloroplast. So, the energy induced from photosynthesis which required for completing the plant life cycle which reduced. The reduction in photosynthetic efficiency has harmful effects on plant growth, development, and reproduction. Above all, the reproduction of cells was negatively affected by high doses of gamma radiation by decreased mitosis and meiosis divisions. So, these effects agree with **Gaafar *et al.* (2017),** who clarified the decrease in shoot and root lengths at higher gamma radiation doses on the basis of reduction in the mitotic activity of plant tissues.

Table 3. Effect of gamma radiation doses on some morphological parameters in Moricandia sinaica plantlets(25 plantlets/ treatments).

Radiation doses / Gy	Bud survival percentage%	Root formation	Mean of buds No.	Mean of shoot No.	Mean of shoot length/cm
Control	96	+++	4.48^{a}	2.28^{a}	2.45^{a}
10	88	+	3.66^{ab}	2.16^{b}	1.64^{b}
20	80	+	3.28^{ab}	1.85^{c}	1.42^{b}
30	60	-	2.33 ^{ab}	1.64^{d}	1.31^{bc}
40	32	-	2.28 ^{ab}	1.32^{d}	1.22^{c}
50	20	-	2 ^{ab}	1^{d}	0.75^{d}
60	12	-	1.2^{b}	1^{d}	0.37^{e}
LSD 5% =			2.63	1.82	0.38

- *Values followed by different letters are significantly difference at = 0.05 level0*
- *+ = Formation of roots - = Deformation of roots*

Genetic diversity revealed by ISSR markers

Several DNA-based markers are now available that can be used to accurately measure genetic diversity in plant species. ISSR markers employ primers based on a repeat sequence, amplifying the sequence between two microsatellites producing several amplification products per primer, which means they are reproducible and cost effective. The genetic diversity between irradiated *Morcindia sinica* plantlets with different gamma radiation doses 0, 10, 20, 30, 40, 50 and 60 Gy is summarized in **Table (4)**.A total of 149 bands were generated from 20 ISSR primers **(Fig. 4)** and all of them are polymorphic bands by a range of 0 to 3 bands per primer with an average of 1.3 fragments per primer. The highest number of bands 11 observed by G-03, while the lowest number was 4 by ISSR 16 with an average of 7.45 bands per primer. The polymorphism percentage varied from 33.33% to 72.7%, with the mean of polymorphism 58.55%. The size of the amplified products ranged from 104 bp (CA) to 1989 bp (ACA). The value of polymorphic information content **(PIC)** was varied from 0.2391(ISSR- 7) to 0.57 (ISSR-2) with an average 0.363 (*PIC*<0.5) classifying most primers as moderately informative **(Yehia *et al.*, 2020).** Primers G-02, CCA, CGA, GT, AG, CA, ISSR 11, ISSR 12 and ISSR 18 recorded the highest PIC (0.371–0.374), confirming their usefulness in detecting genomic variation induced by gamma rays.Marker index value **(MI)** was varied between 0.0176 (G-03) and 0.6449 (ISSR- 2) by an average 0.0681. Nei's gene diversity values **(H)** ranged from 0.227 (ISSR- 7) to 0.6449 (ISSR-2) indicating substantial allelic variation across loci were revealed high genetic diversity, consistent with mutagen induced base substitutions, deletions, or insertions **(Mudibu *et al.*, 2012).** The effective multiplex ratio (E) fluctuated from1.000 (ISSR- 2) to 5.8333 (ISSR 7). High E values point to primers capable of amplifying several informative loci simultaneously, which improves detection efficiency. Equally, the arithmetic mean heterozygosity (H.av) values were 0.0062 (G-03) to 0.0145 (ISSR- 16), signifying that while many polymorphisms existed, they were distributed across a relatively small subset of loci, a characteristic feature of targeted mutagenesis. The discriminating power (D) was higher ranged from 0.3089 (ISSR 7) to 0.8410(G-03) which indicating strong genotype differentiation. Also, resolving power (R) varied from 2.3333(ISSR 7) to 6.7500 (ISSR- 16). High R values indicated primers with strong diagnostic value in mutation breeding programs. ISSR (Inter-Simple Sequence Repeats) are semi-arbitrary markers that can be amplified using PCR with one primer that parallels to a target microsatellite. Additionally, that their amplification does not need information about the genome

sequence and revealed higher polymorphism level than RAPD and AFLP markers according to **Pandotra *et al.* (2013).** The ISSR markers have been demonstrated to be a rapid, simple, dependable, and highly informative method for DNA fingerprinting also ISSR markers afford more truthful genetic polymorphism results so, it used to evaluation genetic diversity, genetic stability, genetic characterization and identification and screening of mutant plants. The impact of g-radiation by increase radiation dose which Changes in the structure of DNA, such as breaks, transpositions, deletions, etc., can lead to the formation of new bands (unique bands) and the removal of existing bands (polymorphic bands). So ISSR analysis showing genetic variation among untreated and irradiated treated plants will be useful in distinguishing variants with differences in morphological characteristics. In the same line with **El-Gazzar *et al.* (2023)** found that gamma radiation led to the appearance of new bands and the absence of others compared to the control plants. Disappearance of ISSR bands in treated plantlets could be mentioned to damages of DNA as single or double-strand breaks, modified bases, oxidized bases, and bulky adducts. Similarly, DNA protein cross links, point mutations and rearrangement of chromosomes induced by gamma irradiation **(Ginchner *et al.*, 2008 and Abhay *et al.*, 2014).** Besides, it has been revealed that free radicals interact with biomolecules as DNA and eliminate electrons from them, which harm to both structure and activity of the DNA. Though, the appearance of new bands could be correlated to the effect of mutation instead of DNA damage. Treated plants with highest doses of gamma rays caused in the highest reduction in content of genomic DNA. Similarly, the appearance or disappearance of bands under gamma irradiation might be considered as molecular markers for radiation processes **(Hussein, 2012).** It has been exhibited that effects of gamma-rays on ISSR fingerprinting might be connected to structural rearrangements in DNA caused by different types of DNA damages **(Mejri *et al.*, 2012).**

Table 4. Amplification results generated by ISSR primers in Moricandia sinaica plantlets.

No.	Primers	TAB	PBN	%PB	BS//bp	PIC	MI	H	E	H.av	D	R
1	G-02	9	0	55.55	215-650	0.3744	0.0290	0.49886	3.6666	0.0079	0.7296	6.0000
2	G-03	11	1	72.7	396-1637	0.3653	0.0176	0.48102	2.8181	0.0062	0.8410	5.6363
3	CCA	9	1	66.66	190-799	0.3734	0.0254	0.49685	3.2222	0.0078	0.7921	5.7777
4	CGA	8	1	62.50	233-787	0.3746	0.0300	0.49936	3.3750	0.0089	0.7720	5.7500
5	GT	9	0	55.55	170-1366	0.3744	0.0290	0.49886	3.6666	0.0079	0.7296	6.2222
6	AG	9	1	55.55	280-1073	0.3734	0.0297	0.49685	3.777v	0.0078	0.7127	6.0000
7	CT	9	0	66.66	388-1056	0.3698	0.0233	0.48979	3.000·	0.0077	0.8202	5.7777
8	CA	10	3	70.00	104-306	0.3717	0.0274	0.49346	3.9000	0.0070	0.6931	5.8000
9	ACA	6	1	50.00	818-1989	0.3657	0.0478	0.48185	4.1666	0.0114	0.6515	5.6666
10	ISSR 2	7	1	57.14	142-722	0.5700	0.6449	0.64499	1.000·	0.6449	0.4444	2.4320
11	ISSR 7	6	1	33.33	142-722	0.2391	0.0385	0.27777	5.8333	0.0066	0.3089	2.3333
12	ISSR 10	5	2	60.00	143-373	0.2904	0.0544	0.35265	5.4000	0.0100	0.4100	3.2000
13	ISSR 11	8	1	62.50	309-1129	0.3721	0.0342	0.49426	3.8750	0.0088	0.6980	6.2500
14	ISSR 12	8	1	62.50	290-959	0.3746	0.0323	0.49936	3.6250	0.0089	0.7363	6.7500
15	ISSR 13	6	3	66.66	168-800	0.3537	0.0491	0.45918	4.5000	0.0109	0.5923	5.0000
16	ISSR 14	6	2	50.00	221-881	0.3360	0.0491	0.42743	4.8333	0.0101	0.5284	4.3333
17	ISSR 16	4	2	50.00	258-956	0.3248	0.0728	0.40816	5.0000	0.0145	0.4973	4.0000
18	ISSR 18	6	2	66.66	594-1288	0.3744	0.0395	0.49886	3.3333	0.0118	0.7793	6.0000
19	ISSR 24	6	2	50.00	309-1252	0.3248	0.0485	0.40816	5.0000	0.0097	0.4947	4.0000
20	G-01	7	1	57.14	422-1242	0.3664	0.0408	0.48313	4.1428	0.0098	0.6547	5.4285
Total		149	26	1171.1		7.269	1.3633	9.3908	78.1355	0.8186	12.8861	102.3576
Mean		7.45	1.3	58.55		0.363	0.0681	0.4695	3.9067	0.0409	0.6443	5.1178

- *Note: Total amplified band (TAB), Polymorphic band no. (PBN), % polymorphic band (%PB), Band size/bp (BS/bp), Polymorphism information content (PIC), Marker index (MI), Heterozygosity index (H), Effective multiplex ratio (E), Arithmatic mean of H (H.av), Discriminating power (D), Resolving power (R)*

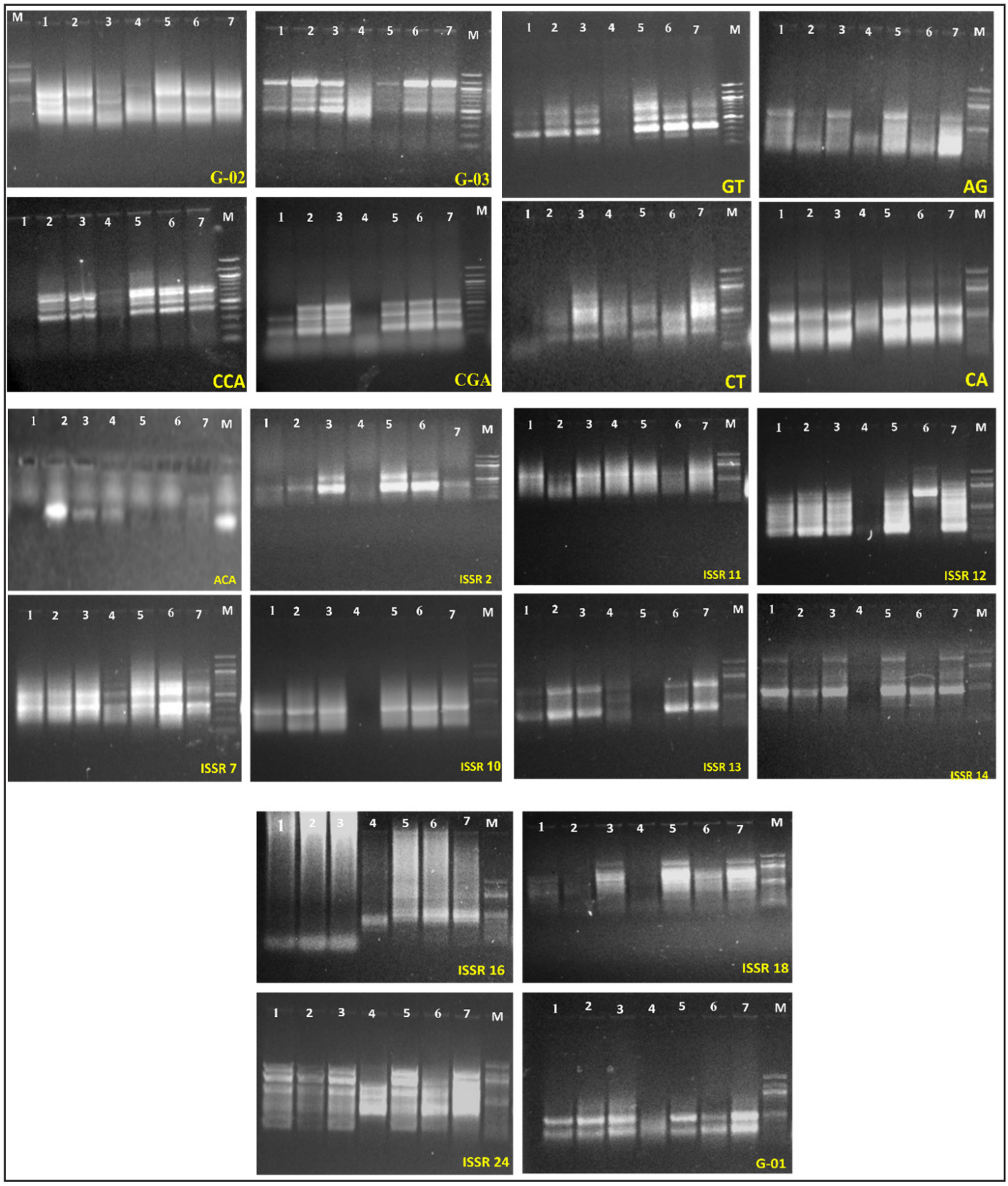

Figure (4): Electrophoresis pattern with 20 ISSR markers of unirradiated and irradiated *Moricandia sinaica* (1) control, (2) Dose 10 Gy, (3) dose 20 Gy, (4) dose 30 Gy. (5) dose dose 40 Gy (6) dose 50 Gy and (7) dose 60 Gy, M = molecular weight marker (1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp)

Determination of organosulfur compounds

Moricandia sinaica is one of xerophytic plants in Egypt. It used in traditional medicine and improve certain disorders such as syphilis, exhibits analgesic, anti-inflammatory, antipyretic, antioxidant, anticancer and antigen toxic properties. However, it has many biologically active components. In this investigation, focused on organosulfur compounds were institute in *Moricandia sinaica* plantlets. Furthermore, study the effect of gamma radiation doses (0,10,20,30 and 40 Gy) by GC- MS. Data in Table 5 resume the result of gamma irradiation on presence or disappearance of some organosulfur compounds. The GC-MS analysis presented that there are13 bioactive organosulfur compounds in both irradiated and non-irradiated samples were recorded. Some compounds being synthesized and others degraded depending on the impact of irradiation. Gamma radiation doses (10, 20, 30 and 40 Gy) have a negative effect on Thiocyanic acid octyl ester, MF: (C9H17NS) and 2-[3-Cyclohexylaminopropylamino] ethylthiophosphate, MF: (C11H25N2O3PS) which disappeared from all irradiated treatments and appear in control only. While the compound 2-Aminoethanethiol hydrogen sulfate (Ester), MF: (C2H7NO3S2) were appeared in all treatments. Dose 40 Gy Participate with control in two compounds are ç-Thionodecalactone,MF: (C10H18OS) and 5à-androstan-16- one cyclic ethylene mercaptole , MF: (C21H34S2),with the proportion of these two compounds in dose 40 Gy being higher than the control. The compound 2-Myristynoyl pantetheine, MF: (C25H44N2O5S) appeared in all treatments except dose 20 Gy with higher proportion than the control. Three compounds tert-Hexadecanethiol, MF:C16H34S, D-fructose, diethyl mercaptal penta acetate, MF: C20H32O10S2 and Cefazolin, MF: C14H14N8O4S3 were appeared with dose 30Gy only. Moreover, another three compounds Leukotriene D4 methyl ester, MF:C26H42N2O6S,Benzo(B)thiophene,2-Ethyl-5,7-dimethyl, MF: C12H14S and 1-(Decylsulfonyl)-1-Deoxyd-Mannitol ,MF: C16H34O7S respectively were disappeared from control and appeared in doses (10, 20 and 40 Gy) respectively. The influence of gamma irradiation on ***Moricandia sinaica*** plantlets organosulfur compounds rehabilitated the composition and the presence of existing substances has pharmacological characteristics, the investigation revealed that, 13 bioactive compounds in both irradiated and non-irradiated samples. So, the data shows that gamma irradiation alters the composition of organosulfur compounds in *Moricandia sinaica* for required plant growth. In this paper, **Table (6)** showed some biological and chemical properties of organosulfer compounds in *Moricandia sinaica* which antioxidant, anti-inflammatory , antifungal, antibacterial and insecticidal activities. Also, the compound D-Fructose, diethyl mercaptal used as A natural sugar used as a sweetener and in food applications. Moreover, the 2- [3-Cyclohexylaminopropyl amino] ethylthiophosphate used as a radioprotective agent and detoxifier of platinum-based chemotherapy agents that helps reduce damage from ionizing radiation. *Moricandia* ***sinaica*** possess valuable phytochemicals as an effective and safe antiangiogenic drugs to be used as a single therapy or in recipe with other chemotherapy agents to treat cancer patients and remedy the complications in diabetic retinopathy, in harmony with **Farooq *et al.*** (**2020**). Sulfur-containing compounds (SCCs) are pivotal secondary metabolites widely distributed in plants, particularly within the Brassicaceae family. Sulfur (S), is considered to be the fourth greatest central nutrient element after nitrogen (N), phosphorus (P), and potassium (K). It plays an important role in the process of plant resistance to biotic and abiotic stress in plants. So, is one of the essential elements for growth, photosynthesis, respiration and the formation of cell membrane structures in plants. However, is a component of amino acids, chloroplasts, sulfatides, vitamins, coenzymes, and prosthetic groups (iron–S clusters, lipoic acid, thiamine and coenzyme A **(Yoshimoto and Saito, 2019; Nakaiand Maruyama-Nakashita, 2020 and Lee *et al.,* 2025**). Because animals cannot synthesize S-containing amino acids and plants are sources of S-compounds as Brassicaceae family vegetables such as broccoli, cabbage, and cauliflower. These vegetables contain characteristic sulfur-containing glucosinolate compounds, which are precursors to bioactive molecules like isothiocyanates and sulforaphane. as well as Liliaceae, like garlic and onion are rich in various forms of sulfur, including sulfides, thiosulfates, and sulfoxides. As a result of their biological functions, such as antioxidant, anti-inflammatory, biocidal, antimicrobial, cytotoxic, a lower risk of developing several types of cancer, neurological disorders like Alzheimer's, and reduced inflammatory bowel disease, mastitis, photoaging, diabetes, cardiovascular disorders, fatty liver disease, and obesity **(Capaldi *et al.,* 2015; Hasanuzzaman *et al.*, 2018; Francioso *et al.*, 2020**). Plants in the Brassicaceae family produce numerous sulfur-containing secondary metabolites, found as a Glutathione (reduced form GSH; oxidized form GSSG) is the major non-protein thiol in plants. It plays a role as a non-enzymatic antioxidant in the ascorbate-glutathione cycle, and participates in many detoxification reactions in plants. Furthermore, GSH is also known as a central regulator of plant signaling during plant–pathogen interactions (**Künstler *et al.*, 2020**). Glutathione is formed by the combination of γ-glutamate and cysteine via γ-glutamylcysteine synthetase (GSH1) through peptide bond and at the same time is combined with glycine catalyzed by glutathione synthase catalyzed by (GSH2) **(Noctor *et al.*, 2012**). Both GSH1 and GSH2 are coded by a single gene and have been observed in Arabidopsis spp. Any alteration in one of the two genes can undergo mutations. GSH biosynthesis is affected by many factors such as sulfur availability and GSH1 activity. Genes

such as GSTM1 and GSTP1 and many more are responsible for the regulation of glutathione metabolism, as these genes produce enzymes that help to maintain homeostasis by glutathione recycling in plants (**Hyman, 2010**).Gamma radiation can alteration in composition of the general metabolic and various biomolecules as sulfur-containing compounds, as a part of biochemical changes caused by the induced stress. In addition to producing both singlestrand DNA breaks (SSBs) and double-strand DNA breaks (DSBs), intrinsic DNA damage may include the loss of a base to form an abasic site, chemical modification of a base to form a miscoding or noncoding lesion, and sugar-phosphate backbone breakage. The accumulation of such damages (unrecognized and unrepaired DNA damage) may cause lethal mutations which in turn can reduce plant genome stability, growth, and productivity and also threaten the organism's immediate survival **(Tuteja *et al.*, 2001).**

Table 5. Gamma radiation effect on organosulfur compounds in unirradiated and irradiated Moricandia sinaica plantlets.

No.	Compounds			Cont.			10Gy			20Gy			30Gy			40Gy		
	Compound name	M.F.	M.W	RT	MF	Peak area%	RT	MF	Peak area%	RT	MF	Peak area%	RT	MF	Peak area%	RT	MF	Peak area%
1	Ethanimidothioicacid,2 (dimethylamin) N[(methylamino carbonyl] oxy]-2-oxo-,methyl ester	C7H13N3O3S	219	5.29	741	**2.36**	8.33	704	**1.24**	4.62	633	**0.65**	5.13	624	1.45	4.74	719	**15.53**
2	Thiocyanic acid, octyl ester	C9H17NS	171	8.81	650	**0.21**	----	----	----	----	----	----	----	----	----	----	----	----
3	2-Aminoethanethiol hydrogen sulfate (Ester)	C2H7NO3S2	157	17.5	744	**0.43**	22.44	736	**1.21**	9.87	664	**1.21**	5.69	612	2.87	6.04	661	**0.34**
4	ç-Thionodecalactone	C10H18OS	186	16.5	688	**0.26**	-----	----	-----	-----	----	-----	-----	-----	-----	12.81	669	**0.67**
5	Benzo[b]thiophene, 2-ethyl-5,7- dimethyl	C12H14S	190	----	----	-----	-----	----	-----	4.68	665	**0.74**	-----	-----	-----	-----	-----	-----
6	tert-Hexadecanethiol	C16H34S	258										43.82	695	4.56			
7	1-(Decylsulfonyl)-1-deoxyd-mannitol	C16H34O7S	370													42.19	635	0.45
8	D-Fructose,diethyl mercaptal	C20H32O10S2	496	----	----	----	----	----	----	----	----	----	5.39	621	4.20	-----	-----	-----
9	5à-Androstan-16-one,cyclic ethylene mercaptole	C21H34S2	350	34.99	713	0.31	----	----	----	----	----	----	----	----	----	12.81	719	0.67
10	2-Myristynoyl pantetheine	C25H44N2O5S	484	24.19	731	**0.34**	4.81	697	**0.90**	----	----	----	41.61	683	1.50	28.11	744	**0.91**
11	Cefazolin	C14H14N8O4S3	454										5.76	713	1.28			
12	Leukotriene D4 methyl ester	C26H42N2O6S	510				22.44	710	1.21									
13	2-[3-Cyclohexy laminopropy lamino] ethylthiophosphate	C11H25N2O3PS	296	5.39	656	**1.19**												

Table 6. Some biological and chemical activities of organosulfer compound in Moricandia sinaica plantlets.

No.	Compound name	Some biological and chemical activities
1	Ethanimidothioicacid, 2 (dimethylamin) N[(methylamino carbonyl] oxy] -2-oxo-, methyl ester.	Oxamyl is a carbamate pesticideis used to control insects, nematodes and acaricides, and mites on various agricultural crops, vegetables, fruits, and ornamental plants. It is not naturally produced by plants. It is a synthetic chemical pesticide which manufactured in labs and applied to crops, where it is absorbed by plant tissues to kill pests that feed on them. Oxamyl is highly toxic to humans and other wildlife through ingestion, inhalation, or skin contact. There is not enough evidence to classify oxamyl›s potential carcinogenicity in humans.
2	Thiocyanic acid, octyl ester	**Glucosinolates** is natural production by plant cells has been observed in specific plants of the Brassicaceae family. It is not a common plant metabolite but rather an end-product of a specific enzymatic reaction.
3	2-Aminoethanethiol hydrogen sulfate	($C_3H_7NO_2S$) is converted into pyruvate, ammonium, and (H_2S) This is one of the main pathways for production (H_2S) in the plant cytosol. Since taurine levels increased in response to osmotic stress. The exclusion of alternate taurine biosynthetic pathways and indicated that taurine likely functions as an osmolyte. This compound is an ester of sulfuric acid and ethanolamine and functions as a biochemical tool, a diuretic, and an anticonvulsant
4	ç-Thionodecalactone.	is a organosulfur analog of the common flavor and fragrance ingredient delta-decalactone ($C_{10}H_{18}O_2$), where the oxygen atom in the lactone ring›s carbonyl group has been replaced by a sulfur atom (a thione group). used in the flavor and fragrance industry for their creamy, fruity, and waxy notes. Thionolactones generally have distinct, often strong, sulfurous aromas.
5	Benzo[b]thiophene, 2-ethyl-5,7- dimethyl.	The benzothiophene core is found in approved drugs like Raloxifene, used for preventing and treating postmenopausal osteoporosis**,** Anti-cancer agents, Anti-inflammatory agents,Anti-microbial agents, Analgesic (pain-relieving) agents, Anti-diabetic and anti-convulsant agents
6	tert-Hexadecanethiol.	is a long-chain thiol and exhibits anti-tumor, antioxidant, antifungal, antibacterial and insecticidal activities in certain plants and organisms. It has been identified as a component in the defensive secretions of the Mupli beetle (Luprops tristis) and found to have anticancer. Additionally, research shows its potential as an antimitotic agent, meaning it may interfere with cell division, and has been linked to the production of nanoparticles with antibacterial properties. It has been identified as a bioactive molecule in plants like *Capsicum annuu*m and has been explored for its potential in developing natural products and chemotherapeutics, particularly due to its antioxidant and antiproliferative effects.
7	1-(Decylsulfonyl)-1-deoxyd-mannitol	Anti-inflammatory and anticancer
8	D-Fructose,diethyl mercaptal	antimicrobial activity, uses as A natural sugar used as a sweetener and in food applications.
9	5à-Androstan-16- one,cyclic ethylene mercaptole	was identified through virtual screening as a potential inhibitor of pancreatic lipase, suggesting it could be used to modulate lipid levels and treat hypercholesterolemia. Its ability to strongly bind to pancreatic lipase, as indicated by a high docking score in in silico studies, positions it as a promising candidate for development into an anti-hypercholesterolemic agent. It may help in modulating lipids in the body, suggesting broader applications in managing lipid-related disorders
10	2-Myristynoyl pantetheine	antioxidant and anti-inflammatory activities. It has been identified as a potential biomarker in exhaled breath for abnormal liver function, and also shows antimicrobial properties. The compound has been detected in red algae and the plant Curculigo orchioides, suggesting its presence in natural products with potential therapeutic benefits
11	Cefazolin	carboxylic acid,is a first-generation cephalosporin antibiotic used for the treatment of a number of bacterial infections. Specifically it is used to treat cellulitis, urinary tract infections, pneumonia, endocarditis, joint infection, and biliary tract infections. It is also used to prevent group B streptococcal disease around the time of delivery and before surgery. It is typically given by injection into a muscle or vein. Cefazolin is in the first-generation cephalosporin class of medication and works by interfering with the bacteria's cell wall.
12	Leukotriene D4 methyl ester	Leukotriene D4 methyl ester is not a widely used compound, but Leukotriene D4 (LTD4) itself is a potent bronchoconstrictor and smooth muscle contracting agent used in scientific research and diagnostic studies for asthma and allergic rhinitis. The methyl ester form may be used as a research chemical or a specific chemical derivative, rather than having direct therapeutic uses, to study the effects of leukotriene D4 on biological systems in a controlled manner.
13	2-[3-Cyclohexylaminopropyl amino] ethylthiophosphate	is a chemical compound, likely a variation of Amifostine used as radioprotective agent and detoxifier of platinum-based chemotherapy agents that helps reduce damage from ionizing radiation

Conclusions

This study demonstrated the different doses of gamma radiation led toalterationsin morphological, molecular, and phytochemical as organosulfer compounds, attributed to alterations in gene expression of *Moricandia sinaica* plantlets. In essence, the plant's altered gene expression is its adaptive mechanism, allowing it to build defenses against radiation-induced damage, a principle used to develop stronger crops to resist adverse abiotic stress**.**

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