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Radio-stimulation of phytohormons and bioactive components of coriander seedlings

[Kişniş tohumlarinda bioaktif maddeler ve fitohormonların ışınlama ile stimulasyonu*]

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ABSTRACT

Objectives: The present work introduces a new study on Egyptian coriander for enhancing total amino acids, some phytohormones (Indol 3- acetic acid, Gibberellic acid and Abscisic acid) and percent volatile oil content by low doses of gamma irradiation.

Materials and Method: Coriander seeds (*Coriandrum sativum L.*) were irradiated with different low doses of gamma-irradiation as 0, 20, 40, 60, 80 and 100Gy and were planted to follow the physiological and chemical components such as some phytohormones, total amino acids and volatile oil content during germination and growth of seedlings.

Findings: Irradiation dose at the range of 60-80 Gy stimulated the fresh and dry weight whereas 100 Gy inhibited the growth. These data were in parallel with increasing Indol-3 acetic acid, Gibberellic acid, Abscisic acid content which increased linearly with applied dose. Also, the same trend was observed with the yield of volatile oil percent. During the same time, irradiation enhanced high percentages of amino acids concentration with 100Gy, which is considered a critical level and the highest low dose for increasing amino acids percent.

Conclusion: These results suggested that low gamma-irradiation dose (80Gy) of coriander seeds could be used as simple techniques to produce seedlings with high quantities of healthy metabolites for human consumption.

Keywords: gamma-irradiation; coriander, stimulation, amino acids, plant regulators

ÖZET

Amaç: Bu çalışma Mısır'da yetişen kişniş bitkisinde amino asit ve fitohormonlar ile uçucu yağların yüzde oranlarını düşük gama ışınları ile artırılması üzerinedir.

Materyal ve Metod: Kişniş tohumu 0, 20, 40, 60, 80 ve 100 Gy olmak üzere farklı düşük doz gama ışımasına tabi tutulmuştur. Bu tohumlar çimlendirme ve filizlerin gelişimi evrelerinde fizyolojik ve fitohormon, amino asit kompozisyonu, uçucu yağ asitleri gibi kimyasal bileşenleri açısından takip edilmiştir.

Bulgular: 60-80 Gy aralığındaki ışıma ile taze ve kuru ağırlık stimüle olurken 100 Gy bu artışı baskıladı. Bu data indol-3-asetik asit, giberellik asit ve absisik asit miktarlarinin artan ışıma dozu karşılığında doğrusal bir artış göstermesi ile uyumludur. Aynı temayül uçucu yağların yüzde veriminde de gözlenmiştir. Aynı zamanda 100 Gy ışıma yüksek oranlarda amino asit konsantrasyonunu arttırdı ve amino asit yüzdesini arttıran en yüksek düşük doz ışıma olarak degerlendirildi

Sonuç: Bu sonuçlar düşük düzey 80 Gy gama ışıması ile basit bir şekilde kişniş tohumlarından yüksek miktarda sağlıklı metabolit içenren filiz tohumu geliştirilebileceğini göstermektedir.

Anahtar Kelimeler: gama-ışıması, kişniş, stimülasyon, amino asitler, bitki regülatörleri

Introduction

The gamma-irradiation (γ) treatment of foods and plant products is nowadays accepted in more than 50 states used as a standard and safe sterilization technique which lowers the risk of microbiological contaminations and prolongs the durability of products [1]. Many international agencies such as International Atomic Energy Agency (IAEA), Food and Agriculture Organization (FAO) and World Health Organization (WHO) have declared that food irradiation up to 10 kGy is safe for human consumption [1,2]. Furthermore, WHO opened this level to 75kGy [3].

Recently, low doses of gamma-irradiation are used for improving plant vigor and yield [4-6]. The stimulation effects of natural irradiation were observed early on the ecological and morphological aspects of some plant species in island community near USA [7]. Now, radio-stimulation effect is more promising for producing some specific compounds [5,8]. In addition, γ-or β irradiation at low doses revealed its stimulatory function on the growth of plants in their early ontogenetic stages [5,9].

Physiological symptoms in a large range of plants exposed to gamma- rays have been described by many researchers [10-12]. The stimulated growth of plants issued from seeds that have been exposed to low irradiation doses was reported [13,14]. However, increasing leaf formation and plant height were markedly enhanced with increasing dose rate in some plant species [15]. Moreover, the irradiation - hormone relationship was also reported [16- 20].

Spices as well as essential oils are derived from fruits and seeds. Coriander (*Coriandrum sativum* L.) is an example that includes the *Umbelliferae*. Coriander has been known for over 3,000 years. Its wild type found in Egypt and the Sudan. It is one of the most important spices food and medicine for Egyptians. UV stimulated the synthesis of volatile oils linalool [21]. Also, increasing gamma irradiation doses increased the concentration of several soluble amino acids of edible seeds [22, 23]. Therefore, the present work aims at stimulating the production of some bioactive and unique organic phytochemicals, as well as studying the physiological changes in seeds after using a safe and simple technique for enhancing some compounds such as total amino acids, some phytohormones (Indol-3 acetic acid (IAA), gibberellic acid (GA) and abscisic acid(ABA)) and percent volatile oil content by low doses of gamma irradiation in Egyptian coriander.

Materials and methods

Irradiation treatments

Coriander seeds (*Coriandrum sativum* L.) were obtained from Agricultural research station at AL Kanater area, near Cairo, Ministry of Agriculture. Dry seeds were irradiated at National Centre for Radiation Research &Technology, Nasr City. Gamma irradiation source was by using Cesium-137 with different doses as 0, 20, 40, 60, 80 and 100 Gy.

Seed planting

The irradiated and un-irradiated seeds were planted in pots and garden soil. The pots were kept in a waterproof net house and watered every alternate day to maintain the sufficient moisture required for the germination of seeds. Growth observations both on the control and irradiated seedlings were recorded 36 days after germination.

Chemical analysis

Determination of phytohormones

Extraction and estimation of phytohormones were carried out according to the method of Unyayar *et al* [24]. Indol 3-acetic acid (IAA), Gibberellic acid (GA), Abscisic acid (ABA) were analyzed. Five gram fresh weight samples were placed in 100 mL methanol: chloroform: 2 N ammonium hydroxide (12:5:3 v/v/v) and homogenized using a Kinematic Polytron homogenizer. After addition 1 µg/100 mL Butylated Hydroxytoluene (BHT), the samples were frozen at -80°C for one week, for further analysis. Then the extracts were transferred into 250 mL conical flasks and added 22.4 mL bi-distilled water. To obtain a homogeneous mixture, the conical flasks were shaken 3 or 4 times. Thus, with the exception of plant growth substances, the other organics in methanol were allowed to pass into the chloroform phase. In all coriander samples, the extraction, purification and quantitative determination of total IAA, GA3 and ABA were done according to literature methods of Unyayar *et al*. (24)

Essential oil content

Essential oil content percent was determined according to British Pharmacopoeia [25] on coriander seedlings using a Clevenger-type apparatus. A weight of 50 g of fresh plant material was submitted to hydrodistillation for 3 hrs. The oil obtained was dried over anhydrous sodium sulphate. The oil percentage was estimated as follows: Essential oil (percent) = Volume of oil in graduated tube (ml) X 100 / weight of sample (g).

Quantitative determination of total amino acids

Total amino acids composition of seeds was determined by amino acid analyzer apparatus model "Eppendorf LC3000" using the method of Stevens et al [26].

Acid hydrolysis: A known weight of coriander seeds powder was defatted with soaking in diethylether overnight to be sure that the sample do not contain any fats and remove pigments and impurities in samples to be clear. A known weight (0.3 g) of defatted plant material received 10 ml 6 N hydrochloric acid in a sealed tube, and then placed in an oven at 110 °C. For 24 hours. Hydrolysates were transferred quantitatively into a porcelain dish and the hydrochloric acid was then evaporated to dryness at 50-60°C on a water bath. Distilled water (5 ml) was added to the hydrolysate and then evaporated to dryness to remove the excess of hydrochloric acid and finally the residue was dissolved in 10 ml distilled water and filtrate through 0.45 mm filter. The filtrate was dried under vacuum with a rotary evaporator, then 10 ml of distilled water were added and the samples dried a second time. One ml of 0.2 N sodium citrate buffer at pH 2.2 was added and the samples stored frozen in a sealed vial until separation of amino acids by amino acid analyzer.

Separation of amino acids by amino acids analyzer:

Samples of amino acids were injected in amino acid analyzer (Eppendorf LC 3000). Each amino acid is separated at specific pH, and then colored by reagent named Ninhydrin. Ninhydrin (triketohydrindene hydrate) is an oxidating agent which leads to the oxidative deamination of alpha-amino groups. It is very important for the detection and the qualitative analysis of amino acids. Ninhydrin also reacts with primary amines however the formation of carbon dioxide is quite diagnostic for amino acids. Alpha amino acids yield a purple substance that absorbs maximally at 570 nm. Imino acids (proline) yield a yellow product (absorption maximum 440 nm). Enhancement effect percentage of amino acid (EE percent) was determined as follows:

[(Amino Acid Content (AAC) of irradiated sample - AAC of control sample)/ AAC of control sample] X 100

Statistical analysis The results were tabulated and analyzed statistically by one-way ANOVA (27). Polynomial regression analysis was performed by using XL.

Results and discussion

The preliminary observations showed that irradiated seeds with 60 Gy to start germination before the control group. The following growth observations either with the control or irradiated seedlings were recorded for 36 days after germination.

Stimulation effect of gamma irradiation on growth parameters (fresh and dry weight)

Figures 1 and 2 show that gamma irradiation increased the fresh and dry weights of shoot coriander particularly up to 60-80 Gy. Both were reduced after these doses reached 100Gy comparing with untreated samples. The relationship between the fresh or dry weight and the applied doses was expressed as polynomial regression analyses. Significant values of correlation coefficient (R^2) were obtained for fresh and dry seedlings 0.6966, 0.9209 respectively. The stimulative effect of gamma-irradiation on growth, especially at low doses was reported in several investigations; exposed seeds of *Cicer arietinum* to gamma rays at doses ranging from 50 to 150Gy a stimulation of branching capacity, fresh weight and dry weight was reported [28]. Similar results were observed in *Atropa belladonna*, in *Cassia angustifalia*, in *Matricaria recutita* and geranium [29-32]. Besides, some researchers found the same trends of stimulation at low doses by gamma-irradiation on different species of plants [4, 9, 33].

Effect of gamma - irradiation on phytohormones

As shown in table 1 and figures 3-5, the irradiation activated producing of IAA and GA speedily at high levels. The promotion of producing IAA, GA has linearity with high significant values of R^2 as 0.9344, 0.9781 for IAA and GA respectively. These results are in harmony with the increased weight of fresh and dry seeds. Due to the increase in the amount of growth hormones increased weight is expected. The rate of increasing GA, IAA per dose was expressed as the slope of the linear equations (Table 1). It is observed clearly, that slope value (concentration of hormone per dose) was very high for GA as 1.8886x and 0.0908x for IAA respectively. At the same time same values were lower and decreased to 0.005x for ABA. This proved the stimulation effects of gamma irradiation for IAA, GA but little bit for ABA. These results explained the promotion effects for IAA, GA in increasing the cell elongation or number of cells both for fresh weight and dry weight respectively. Stajkov G *et.*

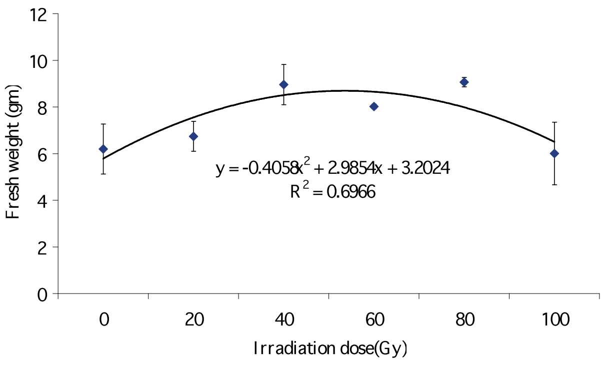

Figure 1. Polynomial curve fit of γ- irradiation effect on fresh weight of coriander seedlings. Each data point represents the mean ± SD; * $p < 0.05$.

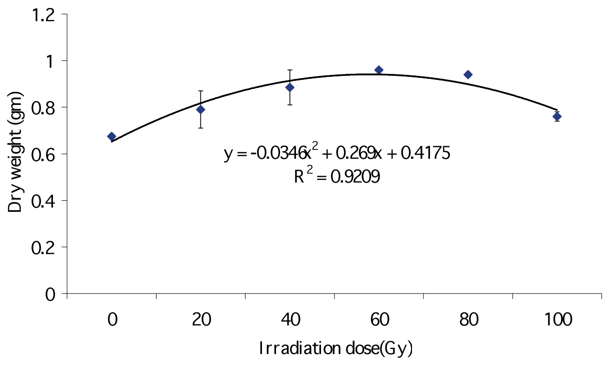

Figure 2. Polynomial curve fit of γ- irradiation effect on dry weight of coriander seedlings. Each data point represents the mean ± SD; $p < 0.05$.

Table 1. Stimulation effect of γ irradiation on phytohormones of coriander seedlings:

.Absicic Acid (ABA)µg/g	Gibberillic Acid (GA)mg/g	Indol-3 acetic acid (IAA)mg/g	Irradiation dose(Gy)
0.06	2.0009	0.0077	Control
0.09	3.7525	0.0087	20
0.18	4.6969	0.0589	40
0.29	6.5799	0.2205	60
0.41	9.3716	0.3138	80
0.55	11.4728	0.42797	100
y=0.005x+0.0119	y=1.8886x-0.2975	y=0.0908x-0.1449	Linear equation
0.9687	0.9781	0.9344	R^2

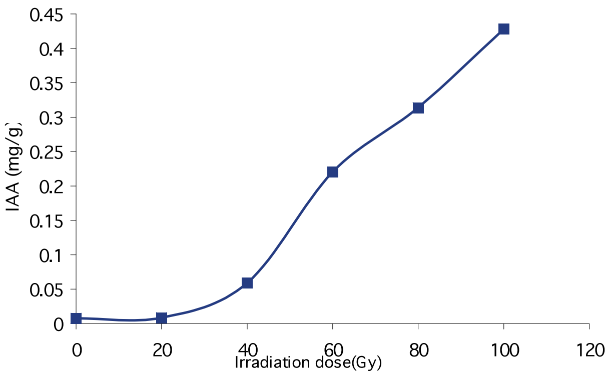

Figure 3. Radio-stimulation of Indol 3- acetic acid (IAA) of coriander seeds.

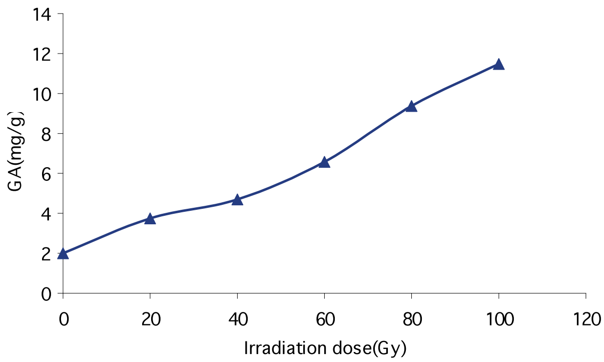

Figure 4. Radio-stimulation effect of gibberillic acid (GA) in coriander seeds.

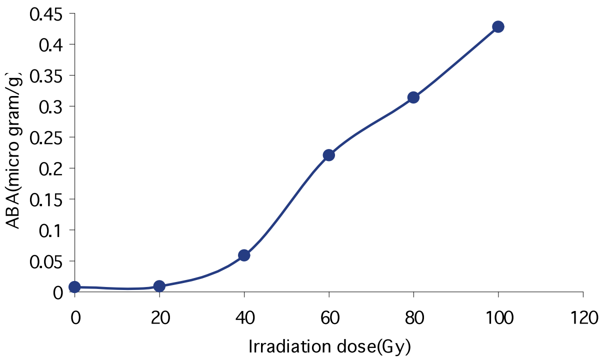

Figure 5. Radio-stimulation effect of absicic acid (ABA) in coriander seeds.

al. investigated the mechanism of the stimulation effect produced by low gamma radiation doses in soybean and indicated that treatment with 5 and 10Gy raised the level and activity of indole auxins and gibberellins in the seeds and plants [34].

Same results were shown by other researchers [4, 35], who suggested that fresh weight increase might be due to higher levels of IAA, GA at low dose which affect the physiology than at genetic level and that role of growth hormones could be crucial. That role of growth regulators enhances the mitotic activity in the meristematic region by increasing the number of dividing cells. However, high doses inactivated GA indirect by an indirect action, probably through reactions with free radicals from the radiolysis of water [36, 37]. Other researchers showed that UV-B irradiation is capable of isomerizing ABA invitro, then playing apart with trace as cistrans-ABA concentration in attenuating its role in the leaf [38]. Also, some work showed that irradiation activates the growth due to promotion of stomatal conductance, transpiration and photosynthetic rate, plant and grain nutritional quality [4].

Effect of gamma - irradiation on the essential oil yield:

Percent of essential oil content of coriander seedlings increased at the range between 40 to 80 Gy in comparison with control samples, as shown in Table 2. This is due to increasing the fresh weight as stimulated by same doses as mentioned before. Studies have been carried out to elucidate the effect of gamma rays on some aromatic plants such as chamomile [31, 39], lemongrass [40], *Mathiola incana* and *Delphinium ajacis* [41], peppermint [42] and Coriander [43].The previous studies report that low doses of γ-stimulation accelerated and increased seed germination, plant growth and coriander oil content plant.

Effect of gamma - irradiation on amino acids composition:

The amino acids were more sensitive to irradiation doses as shown in table 3. The determination of AA composition of shoot proved presence of sixteen, which differ in values as percent in most of tested samples. The effect of irradiation dose as Irradiation enhancement effect (EE percent) was clear with some amino acids after irradiation with 60 Gy. However, using 80 Gy increased most of amino acids when compared to control sample; that dose (80 Gy) only caused EE percent. In the same time, high dose (100 Gy) caused same effect with exception of methionine which decreased sharply at same dose. Therefore, the dose (100 Gy) was considered a critical level which divided the inhibition dose level than promotion dose. To our knowledge, no details have been reported to date in review concerning amino acid content of irradiated seeds during germination. Some researchers showed increase in total protein from 96 percent to 140 percent in irradiated corn after germination. [44]. A significant increase in lysine and valine concentrations at irradiated corn seeds (50Gy) was observed by Iqbal J *et. al.* [22]. This increase may be due to the activation role of low irradiation dose to release amino acids from the bound amino acids composition form. However, high doses decreased the protein content.

Table 2. Effect of γ- irradiation on the essential oil percent of coriander seedlings:

Dose(Gy)	Essential oil percent
Control	0.02
20	0.02
40	0.06
60	0.06
80	0.06
100	0.02

Discussion

Gamma irradiation has been recognized as a reliable and safe method for improving the nutritional value of foods [45]. The stimulating effect of low doses of gamma irradiation on plant growth may be due to activation of cell division or cell elongation, alteration of metabolic processes that affect synthesis of phytohormones or nucleic acids [46]. The explanation of radio-stimulation at low doses may be explained due to indirect role of radiation formation of free radicals [47]. The hydroxyl (HO•) and superoxide anion (O2•-) radicals that are generated by radiation could modify the molecular properties of the proteins and lipids causing oxidative modifications of the proteins and lipid peroxidation (LP) [48]. Another role for stimulation was declared by [19] who suggested that there might be two reasons of radiation effect on

Table 3. Effect of γ- irradiation on amino acids (percent) content of coriander seedlings:

Irradiation dose(Gy)						Amino acid content(percent)
100	80	60	40	20	Control	
1.49	1.43	1.17	0.81	1.12	1.34	1-Aspartic
0.79	0.74	0.62	0.39	0.57	0.71	2-Threonine
0.71	0. 69	0.57	0.33	0.53	0.65	3-Serine
1.72	1. 81	1.40	0.94	1.32	1.6	4-Glutamic
0.89	0.86	0.89	0.44	0.70	0.79	5-Proline
0.79	0.74	0.69	0.46	0.66	0.74	6-Glycine
0.74	0. 91	0.65	0.55	0.61	0.85	7-Alanine
1.27	1.17	0.99	0.65	0.86	1.04	8-Valine
0.05	0.35	0.24	0.05	0.05	0.09	9-Methionine
1.80	1.70	1.39	0.83	1.24	1.58	10-Isoleucine
3.33	3.34	2.57	1.74	2.45	3.06	11-Leucine
0.34	0.31	0.19	0.19	0.19	0.28	12-Tyrosine
2.08	3. 86	2.27	1.23	2.01	2.44	13-Phenylalanine
0.45	0.45	0.39	0.19	0.35	0.35	14-Histidine
1.08	1.01	0.83	0.42	0.78	0.88	15-Lysine
0.71	0.56	0.47	0.23	0.44	0.48	16-Arginin

increasing endogenous hormones: Irradiation causes de novo synthesis of free hormone level to overcome the physical stress, or due to radiation effect, conjugated forms are converted to free form. Concerning low doses of irradiation on proteins can involve fragmentation, cross-linking, aggregation and oxidation caused by oxygen radicals which are generated by water radiolysis [49]. All these reactions depend on the amount of dose which consequently can activate or inhibit the plant growth.

Conclusion

Our study reported that low doses of gamma rays stimulated plant growth, phytohormones, oil production and amino acids content coriander seeds. However, high doses inhibited all these parameters. The radio stimulation effect depends on irradiation source, dose and dose rate according many reports It is necessary to determine the accurate optimum dose for stimulation because of its critical crucial level. These factors explain the inconsistency of some published articles [9]. Moreover, the application of low doses needs preliminary experiments with broad spectrum of doses to nominate the optimum dose for stimulation as shown in our results. Thus using gamma-irradiation at low doses can be a safe fast simple method for improving and stimulating some bioactive and healthy components in coriander seeds.

Conflict of interest

The authors express no conflict of interest.

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