Genetic variability in some economic traits of a sweet basil (*Ocimum bassillicum* L.) cultivar affected by gamma irradiation

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ABSTRACT

The present study aimed to induce mutation in a sweet basil (Ocimum basilicum L.*) cultivar , a medicinal plant, for improving its economic characters .To achieve this purpose , the seeds were exposed to different doses (5, 10, 15 Kr) of gamma irradiation. Irradiated and non-irradiated seeds were cultivated during the two successive seasons 2010 and 2011. The experiment was designed as randomized complete blocks with 4 treatments and 5 replicates. Vegetative characters and chemical composition of essential oil in treated plants were studied. In addition, RAPD-PCR and protein banding profiles using SDS-PAGE were performed for characterization of the irradiated types. The results indicated that 10 and 15 Kr doses had a highly significant impact on essential oil percent, number of branches per plant and plant height in first and second cuts in both seasons compared with control. RAPD -PCR revealed that all primers were polymorphic producing 92 polymorphic bands in total. Protein banding profile demonstrated a conserved band (100 KDa) was present at10Kr in both seasons and a deviated band with high molecular mass (180 KDa) was induced at the same dose. Our results indicated that10 and 15 Kr doses could be useful to produce superior chemical composition and biomass yield in sweet basil plant. In addition, RAPD and protein analysis could help identifying genetic variation among irradiated types.*

***Key words:** Sweet basil, Mutation, Essential oils, RAPD –PCR, SDS-PAGE.*

INTRODUCTION

Basil (*Ocimum basilicum* L.) plants include 50 to 150 species belonging to the family *Lamiaceae*. It can be found in tropical Asia, Africa, Central and South America. *Basilicum* species is widely cultivated commercially as culinary herb for its green and aromatic leaves, which are used dry or fresh as a condiment or for the production of essential oils. Many studies have demonstrated that *O. basilicum* has various beneficial effects on health. The leaves and flowering parts of plant are traditionally used as antispasmodic, carminative, digestive treatments, colic, gastroenteritis, fever, poor digestion, nausea, migraines, insomnia, and diarrhea (Bhattacharier, 1998).

They have been applied externally to treat acne, insect stings, snake bites, skin infections and aromatic (Venancio *et al.*, 2011 and Bora *et al.*, 2011). The increase of world demand for natural products using plants as medicine, and food in addition to, the high cost of pharmaceutical medications have led to an increasing search for alternative medicines to treat and prevent numerous diseases. Thus, there is a need for improving the quality of these natural products or medicinal plants to overcome these problems.Gamma irradiation is now getting recognition throughout the world as a phytosanitary treatment of herbal materials. The biological effect of gamma rays has modified important components of plant cells and affects differentially in morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. It can be useful for production of new mutants with improved commercial characteristics of plants (Kiong *et al*., 2008 and Ashraf *et al.*, 2003). At present, there is a lack of information on the effect of gamma irradiation on sweet basil (*O. basilicum*) plant. Therefore, the present study aimed to induce a mutagenic treatment by gamma rays on sweet basil plant for genetic improvement of its economic characters and composition of essential volatile oils to select economically important types for breeding programs. To achieve this purpose, seeds of sweet basil plant were exposed to different doses (5, 10, 15 Kr) of gamma irradiation. Vegetative growth characters and volatile oils composition as Borneol, 1.8-Cineol, Eugenol, Linalool and Methyl Chavicol were investigated also, RAPD and SDS-PAGE protein analysis techniques were performed for molecular characterization of the irradiated types of plant .

MATERIALS AND METHODS

Gamma irradiation

Three doses (5, 10 and 15 Kr) of gamma irradiation were chosen in the present study based on the previous studies as Rahimi and Bahrani (2011) and Piyanuch and Jarunee (2012). Gamma – rays were generated from the Cobalt-60 source, in Gamma Cell 3500, administrated at the rate of 13.10 rad/sec. installed in the irradiation Laboratory at Middle East Regional Radio – Isotope Center for the Arab Countries at Dokki, Giza, Egypt.

Plant Materials and experimental design

Seeds of sweet basil (*Ocimum basilicum* L.) plants were obtained from Medicinal and Aromatic Plants Department of the Agricultural Research Center, Ministry of Agriculture, Egypt. The experiment was performed as randomized complete blocks design (RCBD) with 4 treatments and 5 replicates and conducted during the two successive growing seasons of 2010 and 2011. The irradiated and non-irradiated seeds (control) were cultivated and harvested two times (cuts) during the two successive seasons.

Planting Seeds

10 g of seeds were used for each dose and sown on February in green house. The seedlings of 2-3 pairs of leaves were transplanted on April in final pots (35cm) containing clay and sand (3:1 V/V). All cultural treatments as nitrogen, potassium and phosphorus fertilizers were performed. Also, the plants were protected from diseases and insects by spraying them with fungicide and insecticides as recommended.

Vegetative growth characters

Vegetative growth characters as plant height (cm), number of the auxiliary branches /plant, leaves fresh and dry weight (g) /plant were recorded in untreated and treated plants in two cuts and both seasons.

Essential oils percentage and chemical composition analysis

Volatile oils were extracted from the leaves of plants as described by British Pharmacopoeia (1963). Analysis of essential oils (Borneol, 1,8- Cineole, Eugenol, Linalool and Methyl chavicol) was studied in second cut of plants in both seasons as described by Gunther and Joseph (1978).

Statistical analysis

The significance of differences among means was carried out using the Least Significant Differences (L.S.D) at p = 0.05.

Molecular analysis

Genomic DNA extraction and RAPD-PCR analysis

Total genomic DNA was isolated from plant leaves using DNA purification E.Z.N.A plant kit (OMEGA bio-tech) according to the manufacturer´s instructions. Four random 10-mer arbitrary primers were randomly chosen, manufactured by Bioligo Co, Korea (Table1) and used to detect polymorphism among the irradiated plants. RAPD-PCR was carried out according to the manufacturer´s instructions for Dream Taq master mix (Fermentase, USA).A matrix for RAPD-PCR was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the lines.

Table (1): Sequence of arbitrary primers assayed in RAPD-PCR.

Primer	Sequence (5´-3´)	TM Value	GC Content%	Annealing temperature
1	CAGGATGACT	71	50	33
2	GCTGACGTAG	81	60	28
3	CATAGTCGAT	64	40	35
4	ACCATGACGT	77	50	41

Determination of protein banding

SDS-PAGE was carried out on protein extracts from irradiated and non-irradiated leaves of plants as described by Laemmli (1970). Electrophoresis was conducted in a vertical slab gel unit (Mini Protein® 3 Cell, Bio-Rad, USA) equipped with a PAC 300 power supply (Bio-Rad, USA).

RESULTS AND DISCUSSION

Plant growth characters, fresh and dry weight of plant

In general, gamma irradiation significantly increased plant height, number of branches, fresh and dry weight of leaves per plant as compared with control (Table 2 and 3). 10 Kr gamma irradiation produced the highest plant height and highest number of branches per plant in both cuts in successive seasons and there were no significant difference between 10 and 15 Kr doses especially in the second season in both cuts as shown in Table (2).Rahimi and Bahrani (2011) reported that the highest plant height was observed at 100 and 200 Gy (10Kr and 20Kr). The stimulating effect of gamma rays on plant growth may be due to stimulation of cell division where there were alterations of metabolic processes that affect synthesis of phytohormones or nucleic acids (Banerji and Datta, 1992). The present results showed that 10 Kr gamma irradiation was more efficient than other doses to increase fresh and dry weight per plant in the first and second seasons in two cuts as shown in Table (3). Kon *et al.*, (2007); and Melki and Salami (2008) observed that low doses of gamma radiation exhibited a significant improvement in dry and fresh weight of chickpea as compared to control plants.

Table (2): Means of plant height and number of branches/plant of sweet basil plants as influenced by gamma irradiation during 2010 and 2011 seasons.

Treatment	Plant height(cm)				Number of branches/plant			
	1st season		2nd season		1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	35.12 d	37.71 d	38.31 C	37.58 C	16.2 b	17.4 c	17.3 c	18.1c
5	43.14 C	41.55 C	41.73 b	42.11 b	16.5 b	17.9 c	19.6 b	19.9 b
10	52.46 a	55.06 a	51.34 a	53.16 a	19.3 a	22.7 a	22.8 a	23.7 a
15	51.22 a	53.27 b	50.31 a	51.30 a	19.1 a	21.4 b	21.9 a	22.3 a
L.S.D 0.05	1.21	2.35	2.47	2.83	0.91	1.03	1.86	1.21

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test
a, b, c, d Means with small superscripts differ significantly

Table (3): Means of fresh weight and dry weight / leaves (g) of sweet basil plants as influenced by gamma irradiation treatments during 2010 and 2011 seasons.

Treatments	Leaves fresh weight/ plant(g)		Leaves fresh weight/ plant(g)		Leaves dry weight/ plant(g)		Leaves dry weight/ plant(g)	
	1st season		2nd season		1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	27.11 b	26.05 c	26.47 b	27.71 c	5.21 d	5.66 c	4.57 c	4.49 c
5	28.07 b	29.15 b	28.56 a	29.07bc	5.85 c	5.50 c	5.05bc	5.12bc
10	30.78 a	31.63 a	30.23 a	32.43 a	6.95 a	7.39 a	6.46 a	6.17 a
15	28.25 b	29.33 b	29.22 a	30.51 b	6.03 b	6.42 b	5.39 b	5.43 b
L.S.D 0.05	2.11	1.95	1.93	1.86	0.58	0.81	0.68	0.75

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test [a, b, c, d] Means with small superscripts differ significantly

Effect of gamma irradiation on volatile oils composition

In general, all doses of gamma irradiation significantly increased volatile oil constituents compared with control. 10Kr gamma irradiation produced the highest volatile oil percentage in 1, 8- Cineole, Eugenol in both seasons also, significant increase was observed in Linalool in the first season and Methyl Chavicol in the second season at the same dose of irradiation compared with control as shown in Tables (4 and 5). Our results are supported by Rahimi and Bahrani (2011) who demonstrated that 100 Gy (10Kr) was the best rate to produce the highest seed oil content.

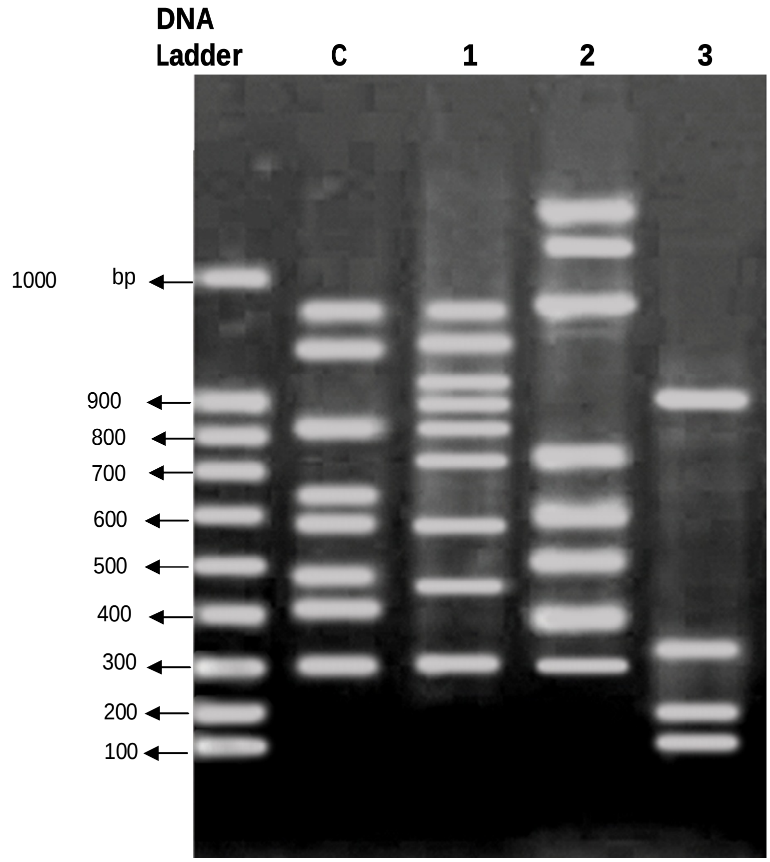

Fig. (1A): RAPD-PCR product using primer (1) where, 1000bp DNA ladder, lane C→control plant, lane 1 → irradiated plant at5 Kr, lane 2 → irradiated plant at 10 Kr and lane 3 → irradiated plant at15 Kr.

Table (4): Means of Major's compounds (%) of sweet basil plant as influenced by gamma irradiation treatments during 2010 season.

	Major's compounds % (1st season , 2nd cut)				
Treatements	**Borneol %**	**1.8-Cineole %**	**Eugenol%**	**Linalool%**	**Methyl Chavicol%**
Control	3.47 d	5.82d	23.61 d	42.01 c	13.20 c
5	3.51 c	5.95 c	24.05 c	42.38 b	13.63 b
10	3.98 a	6.58 a	24.91 a	43.09 a	13.92 a
15	3.91 b	6.34 b	24.79 b	43.07 a	13.90 a
L.S.D 0.05	0.02	0.05	0.11	0.08	0.05

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test a, b, c, d Means with small superscripts differ significantly

Table (5): Means of Major's compounds (%) of sweet basil plant as influenced by gamma irradiation treatments during 2011 season.

Treatments	Major's compounds % (2nd season , 2nd cut)				
	Borneol %	1.8-Cineole %	Eugenol%	Linalool%	Methyl Chavicol%
Control	3.51 c	5.79 d	23.63 d	43.81 d	12.78 c
5	3.72 b	5.81 c	23.75 c	43.41 c	12.85 b
10	3.92 a	6.09 a	24.66 a	43.87 a	13.18 a
15	3.90 a	6.01 b	24.53 b	43.79 b	13.19 a
L.S.D 0.05	0.03	0.06	0.09	0.04	0.06

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test [a, b, c, d] Means with small superscripts differ significantly

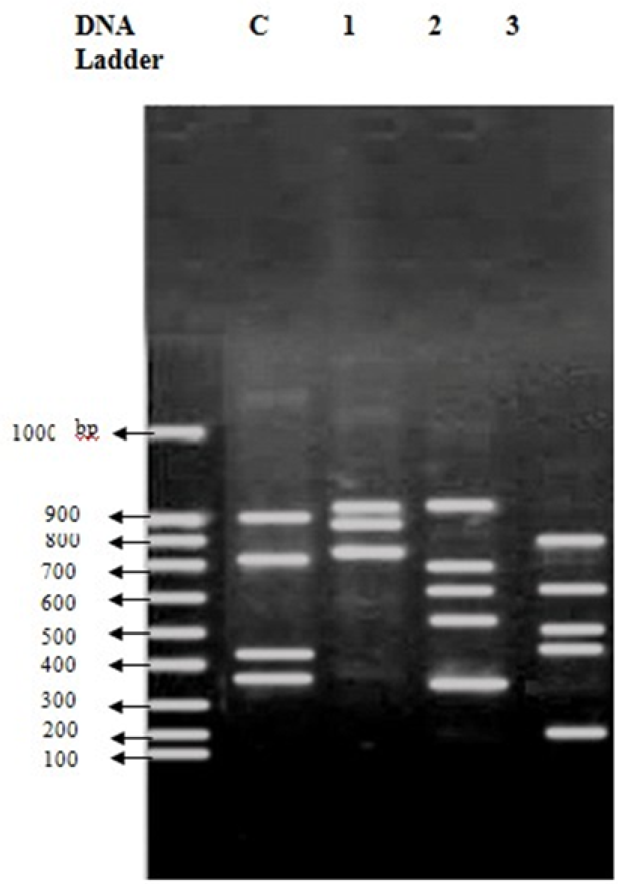

Fig. (1B): RAPD-PCR product using primer (2) where, 1000bp DNA ladder, lane C→control plant, lane 1 → irradiated plant at5 Kr, lane 2 → irradiated plant at 10 Kr and lane 3 → irradiated plant at15 Kr.

Table (6): DNA polymorphism using RAPD-PCR for treated sweet basil plants.

Primer	Total # of amplicons	Monomorphic amplicons	Polymorphic amplicons	%polymorphism /primer
1	29	0	29	100
2	21	0	21	100
3	17	0	17	100
4	25	0	25	100
Total	92	0	92	

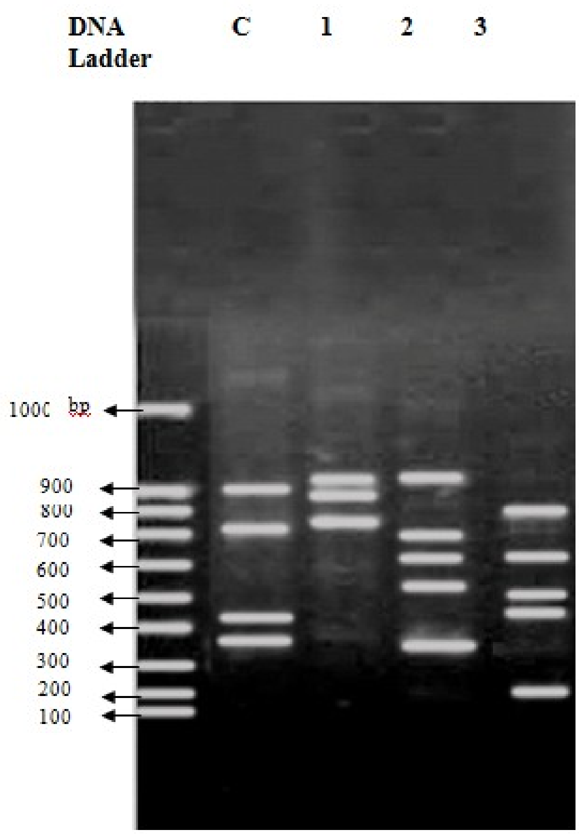

Fig. (1C): RAPD-PCR product using primer (3) where, 1000bp DNA ladder, , lane C→control plant, lane 1 → irradiated plant at5 Kr, lane 2 → irradiated plant at 10 Kr and lane 3 → irradiated plant at15 Kr.

RAPD marker is simple and can amplify short arbitrary oligonucleotide primers without any information of a DNA sequence. It can detect a large number of genetic polymorphism (William *et al.*, 1990). The present results showed that four primers successfully produced 100% percentage of polymorphism with 92 total numbers of fragments in response to treatments with gamma irradiation. Primer 1 amplified the highest number of amplicon (29) while, the least number of amplicon (17) was produced by the primer 3 as shown in Table

(6). The size of amplified fragments varied with the different primers, ranging from 100 to 1000 bp. The main changes in the RAPD profile between irradiated and non-irradiated plants were the appearance or disappearance of different bands with variation in molecular mass and their intensities .These variations may correlate with the dose of gamma radiation on the bases of DNA patterns as shown in Figs. (1A, 1B, 1C and1D). Our results are in accordance with Wendt *et al.*, (2001) who used the RAPD markers to study the effect of gamma radiation on potato. Also, Fadia *et al.*, (2011) found changes in the RAPD profile of the irradiated seeds of *Hibiscus sabdariffa* plants with gamma rays (100, 200, 300, 400, 500, 600, 700 and 800Gy). These variations in DNA may be due to the structural rearrangements in DNA caused by different types of damages as breaks, deletion and transpositions (Danylchenko and Sorochinsky, 2005).

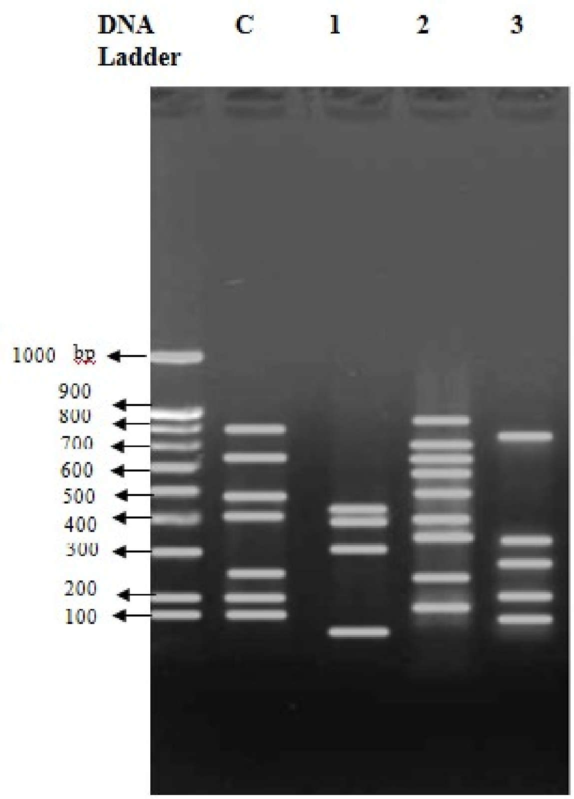

Fig. (1D): RAPD-PCR product using primer (4) where, 1000bp DNA ladder, lane C→control plant, lane 1 → irradiated plant at5 Kr, lane 2 → irradiated plant at 10 Kr and lane 3 → irradiated plant at15 Kr.

Determination of protein banding profile

The results of protein banding patterns revealed that the total number of band was 46 with molecular mass ranging from 180 to 10 KDa. 10 Kr dose enhanced a unique band with molecular mass 100 KDa in the irradiated plants in first and second seasons that was absent in the other irradiated plants at 5,15 Kr and control also, 10 Kr succeeded to induce the highest molecular mass band (180 KDa) in the second season .Both irradiated plants at 10 and 15 Kr exhibited a considerable higher band intensities at180, 130,110, 100, 70, 60, 40 kDa which corresponded to higher amount of denatured protein at these molecular mass as compared to irradiated plants at 5 Kr and control as shown in Fig. (2) . It could be deduced from the results of SDS-PAGE that the protein profile of irradiated and non-irradiated plants varied slightly in number of bands but exhibited higher band intensities compared with control especially at 10and 15Kr. However, major bands could still be observed in both irradiated plants at 10and 15Kr although, the intensities of the bands may differ according to the gamma dosage. Our results are harmony with Abu *et al*. (2005) on cowpea where, gamma irradiation caused an alteration in the protein banding profile.

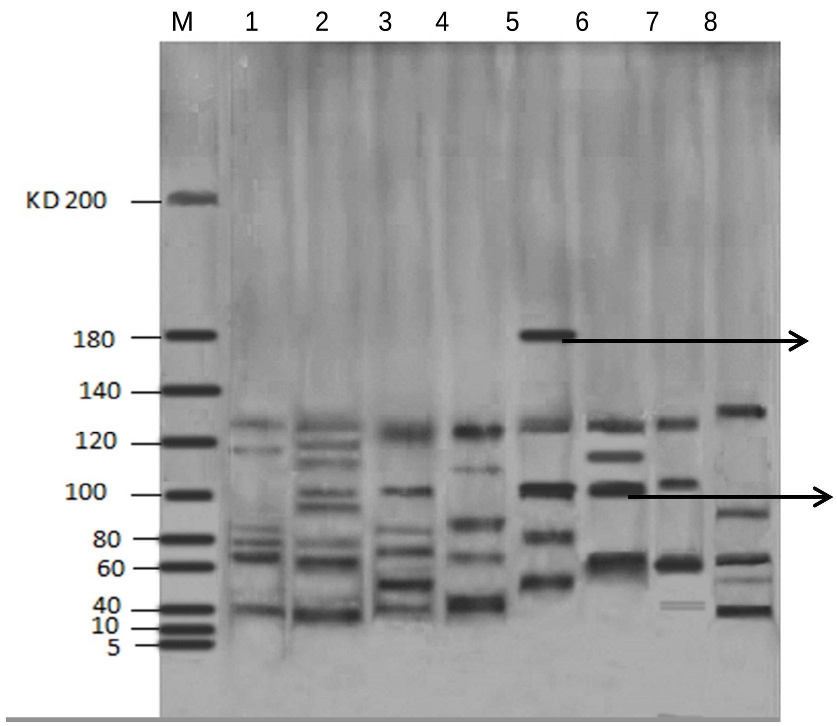

Fig. (2): 12% SDS-PAGE electrophoresis of protein where , (M) 200 KDa protein marker , lane (1-2) control , lane (3-4) treated sample at 5 Kr. dose , lane (5-6) treated sample at 10 Kr. dose and lane (7-8) treated sample at 15 Kr. dose.

Conclusion

The present study suggested that 10 Kr gamma irradiation was the best treatment for stimulating sweet basil plant growth and increasing its volatile oil composition followed by 15 Kr. At the molecular level, agarose gel electrophoresis on DNA and denatured protein could be useful to characterize irradiated plants at three doses of gamma irradiation and instigate a study at the gene level to analyze the modification of certain genes which codes for important cellular protein.

Recommendation

Investigation of gamma irradiation effects on *O. basilicum* could be carried out to produce a mutant with superior qualities for commercial use. In future research, this type of study could be encouraging to screen for a mutant which is able to produce highest amounts of medicinally important metabolites that are useful in the pharmaceutical industry.

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الملخص العربي

الاختلافات الوراثية فى بعض الصفات الاقتصادية لصنف من الريحان الحلو المتاثر باشعة جاما

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الهدف من هذا البحث دراسة تاثير اشعة جاما على نبات الريحان الحلو المنزرع لتحسين بعض الصفات الاقتصادية الهامة مثل طول النبات – عدد الافرع لكل نبات – الوزن الجاف – الوزن الطازج والمركبات الفعالة للزيت و ايضا التوصيف الجزيئى للنباتات المعاملة بالاشعة وذلك باستخدام تكنيك التضاعف العشوائى باستخدام اربع بادئات عشوائية و تكنيك التفريد الكهربى لبروتينات الورقة لنباتات المعاملة بالاضافة لنباتات غير المعاملة (الكنترول). فتم تعريض بذور النبات لاشعة جاما بجرعات٥,١٠,١٥ كيلو راد بمعدل تشعيع ١٣,١٠ راد لكل ثانية.اجريت التجربة الحقلية خلال موسمى الزراعة ٢٠١١-٢٠١٠وصممت التجربة بنظام القطاعات العشوائية الكاملة بثلاث معاملات وخمس مكررات بالاضافة الى الكنترول اظهرت نتائج الدراسة ان المعاملة باشعة جاما ادت الى التاثير على عدد الافرع الخضرية لكل نبات وارتفاع النبات والوزن الجاف والوزن الطازج للنبات و مكونات الزيت الفعالة ايجابيا حيث حدثت زيادة معنوية عند معدل تشعيع ١٠ يليه ١٥ كيلو راد مقارنة بالكنترول. اظهرت نتائج التضاعف العشوائى تفاوت فى عدد الحزم وتعدد الاشكال فى التراكيب الوراثية للنباتات المستحدثةباشعة جاما خاصة عند١٠ و١٥ كيلو راد واظهرت نتائج التفريد الكهربى للبروتين اختلافات فى اعداد الحزم واوزانها الجزيئية ووجدت حزمة متفردة ذات وزن جزيى ١٠٠ كياو دالتون واخرى ذات وزن جزيى عالى حوالى ١٨٠كياو دالتون خاصا عند١٠ كيلو راد .وتوصى هذه الدراسة بانه يمكن استخدام جرعة ١٠ كيلو راد كوسيلة لتحسين بعض الصفات الاقتصادية و مكونات الزيت الفعالة لنبات الريحان الحلو نظرا لاهمية فى الصناعات الدوائية وايضا اهمية استخدام التكنيكات الجزيئية فى التوصيف الجزيئى للتراكيب الوراثية المستحدثة نتيجة التعرض للاشعاع