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Response of Fennel (*Foeniculum vulgare*) to Gamma Irradiation and Gibberellic Acid Treatments

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Abstract: The effect of gamma irradiation and gibberellic acid (GA_3) treatment on different responses of fennel was investigated. Combined effect of γ-irradiation at 2 Krad dose and GA_3 treatment at 50 mgL^{-1} concentration resulted in the maximum germination percentage, seed production, essential oil yield, as well as the best quality of fennel (*Foeniculum vulgare*) oil because of its high content of the flavour compounds, e.g. trans-anethole and fenchone. GA_3 application reduced the harmful effects of higher irradiation doses (5-20 krad).

Key words: Fennel essential oil, *Foeniculum vulgare*, germination, gibberellic acid

Introduction

The medicinal and aromatic plants are of special importance all over the world. The genus *Foeniculum* (family Umbelliferae) includes some varieties among which sweet fennel (*Foeniculum vulgare* var. Dulce) is a perennial plant growing wild in several localities. The main characteristic of fennel oil is the high content of trans-anethole, limonene, estragele, fenchone, γ-terpinene, and α-pinene (Akgul, 1986). Akgul and Bayrak (1988) reported that Turkish bitter fennel have lesser value than sweet fennel because of their low yield of oil, low percentage of trans-anethole and large amount of hydrocarbons.

Gamma radiations have been used in storage, mutation, induction, stimulation, and other purposes. Sjodin (1962) reported that the material and energy necessary for initial growth are already available in the seed, and so the young embryo has no need to form new substances, but only to activate those already stored in the cotyledons. The role of low doses of γ-radiations may be the increasing enzymatic activation and awakening the young embryo, which result in an increasing rate of cell division, which affects not only germination, but also vegetative growth and flowering. Exposing the dry seeds to low γ-irradiation doses resulted in increasing yield of some plants such as safflower (Abo-Hegazi *et al*, 1988), and *Ammi visnage* (El-Shafie *et al*, 1993). Plant hormones play an important role in cell division, elongation, differentiation, and the various metabolic processes, thus could be expected to influence the biosynthesis of essential oils in aromatic plants. Hegazy *et al*., (1990) observed that irradiation of air dry cowpea seeds with 10 Krad dose increased the amount of the growth promoter, Indole- 3-acetonitrile (IAN), as well as the amount of the growth inhibitor, Abscisic aicd (ABA). The effect of irradiation in stimulating the formation of the growth inhibitor was greatly lessened when the seeds were soaked in water before irradiation. Soaking the seeds in GA_3 solution (40 mg L^{-1}) increased the amount of the growth promoter, but was less effective than distilled water in decreasing the amount of growth inhibitor. Atia and Ebaid (1990) also indicated a significant interaction effect of gamma irradiation and GA_3 application to increase the number of open bolls/plant and boll weight of Egyptian cotton.

Therefore, in the present investigation, gamma irradiation and gibberellic acid (GA_3) used individually or in combination for improving yield, seed germination percentage, quality and composition of the essential oil were investigated.

Materials and Methods

The air dried seeds of fennel (*Foeniculum vulgare*) were exposed to different doses of gamma rays (0, 2, 5, 10, 20, 30, 40, 50 Krad) in the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. A preliminary experiment was carried out using a series of concentrations of gibberellic acid (GA_3), cycocel (CCC), and indole-3-acetic acid (IAA) to determine the most favorable plant growth regulating substance. GA_3 (50 mg L^{-1}) was the most effective one.

The field experiment was carried out in the garden of Helwan University. The soil was carefully prepared and divided into plots. Each plot included 3 rows. The seeds were sown in hills (4-6 seeds per hill) at 20 cm distances. After 30 days from sowing, the plants were thinned to one plant per hill. Other cultural practices such as weed control and irrigation were performed whenever it was necessary. The first five plots were subjected to γ−irradiation of 0, 2, 5, 10, and 20 Krad doses, while the second five plots were subjected to the same doses and sprayed with GA_3 (50 mg L^{-1}) two times. The first spraying was applied after 45 days from sowing, and the second at the flowering stage (90-d-old) by an atomizer until dripping the treated plants. Tipple was used as a wetting agent in order to minimize the surface tension between leaves and growth regulating substance. The spraying process was always performed early in the morning. After about seven months, the fruits were harvested before full ripening. The plants were cut off at the soil surface and left in a shaded place about two weeks, then hammered for fruit separation. Seed germination percentage was expressed as:

$$\text{Germination Irradiation Index (GII)} = \frac{\text{Germination of irradiated seeds}}{\text{Germination of control seeds}} \times 100.$$

Yield production was assessed as number of umbels/ plant, fresh and dry mass of umbels/ plant, weight of 100 seeds, percentage of oil content, and oil yield/ 100 plants.

Statistical analysis was carried out according to Snedecor and Cochran (1980) using analysis of variance (Completely Randomized three-factor Design) and the significance was determined using L.S.D. values at p= 0.05 and 0.01.

Electrophoretic protein banding was examined in pre-sowing gamma-irradiated and unirradiated seeds, as well as in the seeds of plants developed from 20 Krad γ-irradiated seeds in comparison with control un-irradiated seeds. Separation of proteins was performed using Sodium Dodecyl Sulphate

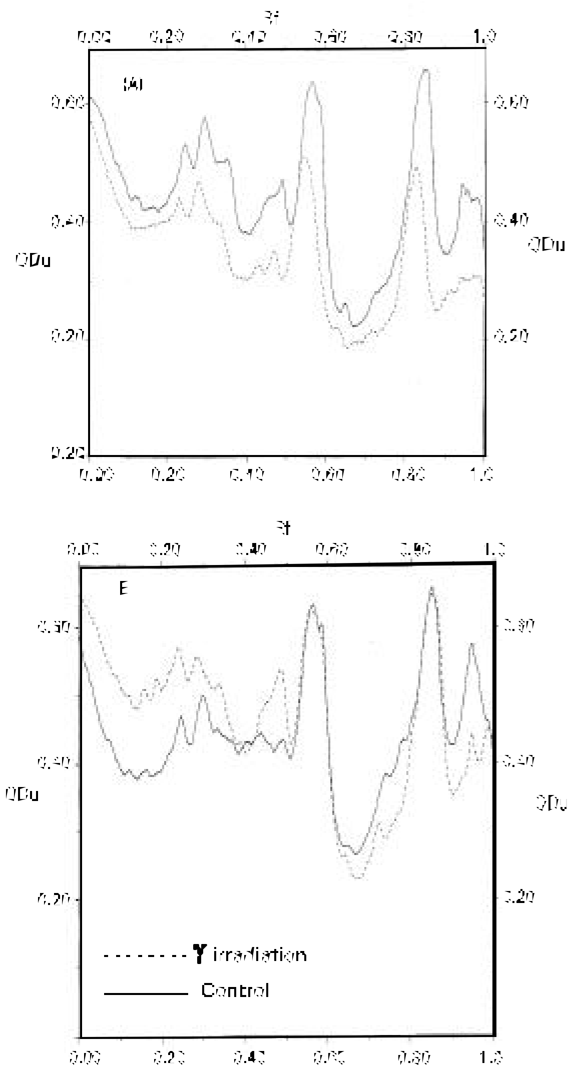

Fig. 1: Protein profile of A: the pre-sowing γ- irradiated seeds, and B: the produced seeds by fennel plants.

Polyacrylamide Gel Electrophoresis (SDS- PAGE), in the central laboratory of Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

The seed content of essential oil was determined by steam distillation according to the method of Guenther (1961). The amount of oil obtained from plant material was calculated according to the following equation: Oil % = Observed volume of oil in graduated tube (ml)/ weight of sample (g.)x 100. The yield of oil produced/plant was calculated by multiplying the average of fresh weight of seeds/plant by the average of oil percentage/plant for different treatments. GLC Model HP- 5890 Gas- Liquid Chromatograph equipped with flame ionization detector was used for the separation of essential oil components, in the National Center of Research, Cairo, Egypt.

Results and Discussion

Germination percentage of fennel seeds under irradiation conditions was expressed as Germination Irradiation Index (GII) (Table 1). The data reveal a stimulatory effect of gamma radiations at 2 Krad dose on germination of fennel seeds by 2% as compared with un-irradiated seeds. Higher doses of gamma rays exhibited a gradual retarding effect on seed germination to become 0.7% of the control at 50 Krad treatment. GA_3 application led to a marked rise in germination percentage of un-irradiated and γ-irradiated seeds up to 20 Krad treatment, above which there is no valuable effect of GA_3 on germination. The highest germination percentage of fennel seeds was recorded for 2 Krad γ-irradiated seeds, treated with 50 mg L^{-1} of GA_3; followed by GA_3- treated un-irradiated seeds, and 2 Krad γ–irradiated seeds. The positive effect of GA_3 application on seed germination may be due to its effect on enzymatic activity and synthesis of RNA and protein (Jacobson *et al*, 1979), as well as on the endogenous phytohormones (Hegazy *et al.*, 1990).

Table1 shows that gamma irradiation doses of 5, 10 and 20 Krad were accompanied by a progressive decrease in number of umbles per plant, as well as the fresh and dry mass of umbles. Nevertheless, the pre-sowing treatment with 2 Krad dose of gamma rays resulted in increasing the number of umbels produced per plant, fresh and dry mass of umbels and seed mass. Application of GA_3 resulted in a considerable improvement in quality of the produced seeds, as well as the number, and mass of the fresh and dry umbels per plant, particularly at 2 Krad γ–irradiation treatment. Georgieva (1987) attributed the inhibitory effect of high doses of γ–irradiation on yield to the enhanced effect on peroxidase and polyphenoloxidase activity, and the increased content of quinones which may affect cytochrome oxidase activity, pollen germination, and pollen tube growth.

Thegene changes during irradiation might be reflected in the storage protein of the plants, as the seeds are enriched with such altered proteins. The present investigation is concerned with the qualitative and electrophoretic studies of protein profiles in control and pre-sowing 20 Krad γ–irradiated fennel seeds as well as in the produced seeds (Table 2 and Fig. 1). The protein bands showed a wide variation in 20 Krad treated seeds as compared to control. The control seeds showed 23 bands with mobility from 0.011 to 0.974. The molecular masses ranged approximately from 269.113 to 11.761 KDa. In the pre-sowing γ-irradiated seeds, some bands were missing, like those with molecular masses of 269.113, 109.896, 99.317, 43.254, and 15.797; meanwhile some new bands appeared with molecular masses of 220.085, 117.721, 94.787, 40.577, and 13.433. The notable changes in the protein profile of 20 Krad γ-irradiated seeds were the subtraction of protein having high molecular weight and addition of new low molecular weight protein. This may be due to the partial degradation of protein by the high γ–irradiation dose. The produced seeds by control plants showed 24 bands, and the mobility ranged between 0.075 and 0.978 (approx. mol. mass 140.528 to 11.65 KDa). The seeds produced by plants which developed from pre-sowing 20 Krad γ-irradiated seeds, showed 25 bands. Some of bands disappeared as compared to control, such as those with molecular masses of 103.296, 75.678, and 39.794, while some new bands appeared with molecular masses of 230.008, 101.221, 17.532, and 13.241. Except one new protein with high molecular weight (230.01 KDa), the appearance of proteins having relatively lower molecular

Table 1: Effect of γ-irradiation and GA_3 treatment on germination, essential oil percentage (A: in the pre sowing γ-irradiated seeds, B: in the produced seeds) and essential oil yield (ml/100 plants) of fennel

Treatment		Germination irradiation Index (GII)	Yield				Essential Oil Percentage (%)		Essential oil yield (ml/100 plants)
GA_3	Irradiation (Krad)		No. of umbels $Plant^{-1}$	Umbel fresh mass (g $Plant^{-1}$)	Umbel Dry mass (g $Plant^{-1}$)	Seed mass (g $Plant^{-1}$)	(A)	(B)	
	0	100	99.16	124.34	69.90	3.09	0.65	0.74	92.01
	2	102	120.36	154.11	74.31	4.34	0.65	0.81	124.82
	5	87	81.20	95.20	46.55	2.15	0.65	0.58	55.21
Untreated	10	66	67.51	86.36	27.97	1.20	0.65	0.39	33.68
	20	55	51.66	64.54	2.61	0.91	0.65	0.32	20.65
	30	8.5	0	0	0	0	0.65	0	0
	40	1.3	0	0	0	0	0.65	0	0
	50	0.7	0	0	0	0	0.65	0	0
	0	103	109.71	135.19	74.23	3.81	0	0.79	106.80
	2	105	134.13	168.14	86.54	5.11	0	0.87	146.28
	5	93	90.12	102.24	53.22	2.72	0	0.66	67.47
Treated	10	74	77.19	94.45	33.46	1.63	0	0.45	42.50
	20	65	60.16	71.15	24.03	1.41	0	0.37	26.30
	30	9.5	0	0	0	0	0	0	0
	40	1.3	0	0	0	0	0	0	0
	50	0.7	0	0	0	0	0	0	0
L.S.D.	0.05	1.3	8.34	6.80	4.83	0.45	0	0.05	4.83
L.S.D.	0.01	1.7	9.11	7.12	5.59	0.62	0	0.07	5.78

Table 2: Protein profile of A: the pre-sowing γ-irradiated seeds, and B: the produced seeds by fennel plants in response to 20 Krad γ-irradiation treatment

(A)						(B)					
Band No	Control		Band No	γ-irradiation (20 Kard)		Band No	Control		Band No	γ-irradiation (20 Kard)	
	Relative Front (Rf)	Mol. Mass Kda		Relative Front (Rf)	Mol. Mass Kda		Relative Front (Rf)	Mol. Mass Kda		Relative Front (Rf)	Mol. Mass KDa
1	0.011	269.113	1	0.031	220.085	1	0.075	140.528	1	0.027	230.008
2	0.106	109.896	2	0.093	117.721	2	0.119	103.296	2	0.075	140.528
3	0.128	99.317	3	0.137	94.787	3	0.162	81.885	3	0.124	101.221
4	0.17	77.866	4	0.172	76.852	4	0.175	75.678	4	0.162	81.885
5	0.196	66.532	5	0.196	66.532	5	0.219	63.076	5	0.212	64.014
6	0.225	62.22	6	0.219	63.144	6	0.241	60.045	6	0.246	59.457
7	0.247	59.237	7	0.247	59.237	7	0.294	53.352	7	0.294	53.352
8	0.291	53.693	8	0.291	53.693	8	0.327	49.554	8	0.332	49.068
9	0.34	48.191	9	0.338	48.429	9	0.338	48.348	9	0.347	47.405
10	0.389	43.254	10	0.417	40.577	10	0.361	46.025	10	0.392	42.959
11	0.413	40.978	11	0.433	39.206	11	0.398	42.329	11	0.447	37.983
12	0.483	35.016	12	0.47	36.064	12	0.427	39.794	12	0.782	35.105
13	0.558	29.629	13	0.545	30.516	13	0.447	37.983	13	0.555	29.84
14	0.581	28.208	14	0.563	29.339	14	0.478	35.453	14	0.577	28.406
15	0.636	24.948	15	0.625	25.568	15	0.551	30.135	15	0.639	24.748
16	0.667	23.289	16	0.664	23.404	16	0.573	28.687	16	0.69	22.098
17	0.713	21.006	17	0.700	21.635	17	0.639	24.748	17	0.717	20.83
18	0.764	18.762	18	0.737	19.901	18	0.686	22.316	18	0.741	19.732
19	0.792	17.601	19	0.819	16.593	19	0.726	20.424	19	0.77	18.508
20	0.841	15.797	20	0.914	13.433	20	0.768	18.599	20	0.794	17.532
21	0.936	12.789	21	0.929	12.979	21	0.836	15.966	21	0.838	15.888
22	0.947	12.478	22	0.940	12.664	22	0.939	12.793	22	0.920	13.241
23	0.974	11.7611	23	0.974	11.764	23	0.947	12.482	23	0.936	12.793
						24	0.978	11.65	24	0.949	12.42
						25	-	-	25	0.978	11.65

Table 3: GLC analysisof essential oil components of A: the pre-sowing γ-irradiated seeds, and B: the produced seeds by fennel plants in response to γ-irradiation and GA_3 treatment

Compounds %	(A)			(B)					
	γ-irradiation (Krad)			γ-irradiation (Krad)			γ-irradiation (Krad)		
	0	2	20	0	2	20	0	2	20
α-pinene	0.4112	0.1373	0.6527	0.4679	0.3091	0.6634	0.4135	0.3038	0.6048
α-phellandrene	0.4063	0.2404	0.5212	0.4637	0.3445	0.5912	0.4208	0.3047	0.5021
Limonene	2.1924	1.0512	0.4335	2.3415	1.1145	04.7321	2.102	1.0674	4.3213
Total hydrocarbons	0.0099	1.6089	0.6074	3.2731	1.7681	5.9867	2.9363	1.6759	5.4282
Fenchone	0.2255	5.7314	2.0071	3.2108	5.0326	2.078	3.3691	4.2344	2.8342
Methyl-chavicol	4.3211	5.1244	2.98	4.2122	5.1022	2.6641	4.7232	5.2234	2.7711
trans-anethole	80.4978	84.361	76.3117	81.1502	85.1502	78.1047	83.0464	86.0092	79.6735
Anis-aldehyde	0.7612	0.9873	0.4344	0.8403	1.0133	0.4322	0.8907	1.0947	0.4755
Total oxygenated compounds	88.8056	96.2041	81.7332	89.4135	96.2983	82.279	92.0294	96.5617	85.7543
Not identified	8.1845	2.187	12.6594	7.3134	1.9336	10.7343	5.0343	1.7624	8.8175

weight was the marked feature in the produced seeds after the pre-sowing treatment with 20 Krad dose of gamma rays. This may reflect genetic changes during irradiation with high doses. Afify and Shousha (1988) attributed the changes in protein patterns after γ–irradiation to partial protein degradation, scission of peptide and disulfide bonds and addition to aromatic and heterocyclic amino acid residues. Similar assumption has been suggested by Booth (1970) and Desrosier (1970).

The essential oil content of fennel dry seeds was not affected by the pre-sowing exposure to gamma rays, recording the same value (0.65%), at different doses used (Table 1). Exposing the fennel seeds to pre-sowing gamma irradiation was beneficial at 2 Krad dose, which markedly increased the essential oil percentage and enhanced yield of essential oil/ 100 plants, may be due to its stimulative effect on fresh mass of umbels and seeds, as well as the activation of enzymes involved in the metabolism of essential oil formation.

The essential oil collected from pre-sowing 20 Krad γ–irradiated seeds, as well as from the seeds by plants developed from seeds subjected to the same treatment was analyzed using Gas Liquid Chromatography (GLC), in comparison with that of the controls (Table 3). The identified flavour components of fennel oil could be generally classified into two categories, namely hydrocarbons (α-pinene, α-phellandrene, and limonene) and oxygenated compounds (fenchone, methylchavicol trans-anethole, and anisaldhyde). Trans-anethole represent the major component, followed by fenchone and methylchavicol. The highest level of total oxygenated compounds (96.56%) and lowest content of hydrocarbons (1.67%) were detected by the combined effect of GA_3 (50 mg L^{-1}) and gamma irradiation (2 Krad). Meanwhile, the highest level of total hydrocarbons (5.98%) and lowest level of oxygenated compounds (83.27%) were recorded in plants treated with 20 Krad γ-irradiation dose. The obtained results agree with that of Guenther (1961), who reported that the main components in fennel seed oil are anethole and fenchone, which define the oil quality. Accordingly, the best quality of the essential oil of fennel seeds was obtained by 2 Krad γ–irradiation treatment combined with GA_3 application because of their high content of trans-anethole and fenchone, and low content of α–pinene, α-phellandrene and limonine, as a result of these treatments. Similar results have been obtained by El-Geddawy and Rashwan (1993), and Farag *et al.*, 1996) who reported that the fruit oil with a high percentage of oxygenated compounds and low amount of hydrocarbons seems to be a valuable flavoring agent for various food products.

Reference

Abo- Hegazi, A.M.T., A.I. Ragab and A.K. Moustafa,1988. Heritability and genetic variability for some characters of sunflower in M_3 and M_4 generation after irradiation.– Minufiya J. Agric. Res., 13: 3- 15.

Afify, M.R. and M. Shousha, 1988. Effect of low dose irradiation on soybean protein solubility, trypsin inhibitor activity and protein patterns separated by polyacrylamide gel electrophoresis.- J. Agric. Food Chem., 36: 810- 813.

Akgul, A., 1986. Studies on the essential oils from Turkish Fennel seeds (*Foeniculum vulgare* M. var Dulce).- Progress In Essential Oil Res., 487 – 489.

Akugul, A. and A. Bayrak, 1988. Comparative volatile oil composition of various parts from Turkish bitter fennel (*Foeniculum vulgar* var. vulgare).- Food Chem., 30: 319-323.

Atia, Z.M.A. and M.A. Ebaid, 1990. Effect of gamma irradiation and gibberellic acid on yield, components and fiber properties of Egyptian cotton. – Minufiya J. Agric. Res., 15: 1- 13.

Booth, M.R., 1970. Modification of wheat gliadin by gamma rays. – J. Sci. Food Agric., 21: 145- 153.

El-Geddawy, M.A.H. and M.R.A. Rashwan, 1993. Effect of gamma irradiation on flavor components of three Egyptian spices. – Assiut J. Agric. Sci., 24: 113- 124.

El-Shafie, S.A., M.M. Mazrou, S.A. El-Kholy and S.A. Sayed, 1993. Physiological influence of pre-sowing gamma irradiation on the growth, drug yield and some chemical constituents of *Ammi visnaga* L. plants. – Minufiya J Agric. Res., 18: 2565- 2578.

Farag, Z.S., N.H. Aziz and A. Ali, 1996. Comparing effects of washing, thermal treatments and gamma irradiation on quality of spices. – Nahung. 40: 32- 36.

Georgieva, I.D., 1987. Cytochemical investigation of pollen tubes after gamma irradiation. II. Effect of the irradiation on quinone formation. Phytomorphol., 37: 159- 163.

Guenther, E., 1961. The essential oils.- Vol. IV Van Nostrand Comp. Inc. New York.

Hegazy, A.T., H.A. Moursi and E. Youssef, 1990. Hormonal picture of cowpea seeds as affected by gamma irradiation and soaking in water or GA_3 solution. Egypt, J. Physiol. Sci., 14: 19- 34.

Jacobson, J.V., T.J.V. Higgins and J.A. Zwar, 1979. Hormonal control of endosperm function during germination.- The Plant Seed, eds. I. Rubenstein, B.G. Gegenbach, R.L. Philips and C.E. Green, Academic Press, New York, Pp. 241 – 262.

Sjodin, J., 1962. Some observations in X_1 and X_2 of *Vicia faba* L. after treatment with different mutagenes.- Hereditas, 48: 565- 573.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods.- 6th Ed., Iowa, State University Press, Ames.