

Effect of Gamma Irradiation on Qualitative and Quantitative Characteristics of Canola (*Brassica napus* L.)

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Abstract: In order to study the effect of cobalt 60 gamma irradiation on agronomic characteristics and quality of fatty acids of canola, an experiment was conducted by using split-plot on the basis of randomized complete block design with four replications in Agricultural Research Station of Islamic Azad University of Yasouj during 2009-2010. Main plots consisted of Tallaye and Okapy cultivars and sub plot were six levels of gamma irradiation including zero (control), 100, 200, 300 400 and 500 Gy. The results of this study showed that plant height, harvest index, 1000-seed weight, seed yield, seed oil percent, linolenic, linoleic and oleic fatty acids were affected with different levels of irradiation. With increasing irradiation up to 300 Gy the measured traits decreased and the minimum amounts were obtained in 500 Gy level. The highest seed yield (3985kg ha⁻¹), oil percent (48.3 %), linolenic acid (11.5 %), 1000 seeds weight (5.3 g), harvest index (31), plant height (94 cm) were obtained in 100 Gy level. The highest (3998 kg ha⁻¹) and the lowest (1545.81 kg/ha) seed yield were achieved in 100 Gy and Tallaye cultivar and 500 Gy and Okapi cultivar, respectively. Talayeh cultivar with 200 Gy and Okapi cultivar with 500 Gy produced the highest and the lowest oil percent, respectively. The highest (12.7 %) and the lowest (7.6 %) linoleic acid were also obtained in 200 Gy and Okapy cultivar and 500 Gy with Tallaye cultivar, respectively.

Key words: Canola · Gamma rays · Fatty acids · Agronomic characteristics

INTRODUCTION

Until recently, rapeseed was a relatively unimportant crop. But as soon as plant breeders were able to get rid of two undesirable substances, rapeseed started to become more interesting. Today it is grown not only as a raw material for biodiesel, industrial oils and lubricants; it is also used as a source of cooking oil for margarine production.

Canola (*Brassica napus* L.) is an important oil crop, ranking third only to soybean and palm oil in global production. It is a member of the family Brassicaceae (syn.Cruciferae). It is a winter or spring crop and is amenable to growth in cooler climates. Once considered a specialty crop for Canada, it is now a global crop. Many other countries including USA, Australia and those in Europe also grow canola. However, Canada and the United States account for most of the canola crop [1]. Its oil also has potentially developed in the biodiesel market. In addition to oil production, the leaves and stems of

canola provide high quality forage matter because of their low fiber and high protein content [2] and can be milled into animal feeds [3].

Canola is a specific type of rape seed associated with high quality oil and meal. It has less than 2% erucic acid and its meal has less than 30 μmole of glucosinolates. It contains 40-45% oil and 36-40% protein. Oil and meal are now very acceptable as alternatives to soybean oil and meal [4,5]. Canola grows well in dry environments and can tolerate moderately saline soil conditions [3, 6].

Pre-sowing seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition [7, 8]. Studies have been carried out to elucidate the effect of gamma rays on some aromatic plants such as chamomile [9], lemongrass [10], *Mathiola incana* and *Delphinium ajacis* [11] and peppermint [12].

Gamma rays generally influence plant growth and development by inducing cytological, genetically, biochemical, physiological and morphogenetic changes in

cells and tissues [13]. There are some reports which showed that the higher exposures of gamma rays were usually inhibitory [14-16], whereas lower exposures were sometimes stimulatory [17-23]. Gamma rays are the most energetic form of electromagnetic radiation, possesses the energy level from 10 kiloelectron volts to several hundred keV and they are considered as the most penetrating in comparison to other radiation such as alpha and beta rays [24]. Gamma rays belong to ionizing radiation and interact on atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds [25, 26].

Several positive mutations have been created in agricultural crops by using gamma irradiations. Crops with improved characteristics have successfully been developed by mutagenic inductions [27-29] developed a high yielding barley variety with early maturity, high protein contents and stiff straw by mutation breeding techniques.

Khatri *et al.* [30] collected three high grain yielding and early maturing mutants by treating seeds of *Brassica juncea* L. cv. S-9 with gamma rays (750-1000K Gy) and EMS.

Shah *et al.* [31] developed a new oil seed *Brassica napus* L. cv. ABASIN-95 by induced mutation. They exposed seeds of *B. napus* L. cv. Tower to 1.0, 1.2 and 1.4 K Gy gamma rays and the resulting new variety was high yielding, resistant to Alternaria blight and white rust.

The objective of the study was to investigate the effects of pre-sowing gamma rays (Cobalt 60) the seeds of canola (*Brassica napus* L.) on agronomic characteristics and fatty acids.

MATERIALS AND METHODS

This experiment was conducted in Agricultural Research Station of Islamic Azad University of Yasouj, Iran (51°41' E, 30°50' N, 1831.5 m height from sea level) during 2009-2010. The experiment was a split plot based on a complete randomized block design with four replications. Main plots, canola cultivars, were V1=Tallaye and V2=Okapy, obtained from the Seed and Plant Improvement Institute in Iran. Sub plot, cobalt 60 gamma

Table 1: Soil physical and chemical characteristics of the experimental site

Variable	
Texture	Silty-Clay
pH	7.85
EC dS m ⁻¹	1.0
Organic matter (%)	0.69
N(%)	0.076
P mg Kg ⁻¹	16
K mg Kg ⁻¹	206
Fe	5.4
Zn	0.1
Mn	9.9
Cu	0.98

irradiation, were irradiation at rates of G1=zero (control), G2=100, G3=200, G4=300 G5=400 and G6=500 Gray (Gy). Seeds of canola (*Brassica napus* L.) were surface sterilized with 1 % Calcium hypochloride, dried under laminar flow hood. The seeds were then exposed to radiations with 60 Cobalt emitting gamma rays with time periods of 2.46, 4.92, 7.38, 9.85 and 12.31 minutes for 100, 200, 300, 400 and 500 Gy, respectively. Seeds were irradiated at the Atomic Energy Organization of Tehran, Iran. To determine the soil characteristics, 15 samples from 30 cm depth were collected and analyzed by the Yasouj Soil Testing Laboratory for basic soil physical and chemical properties (Table 1). One hundred and fifty kg ha⁻¹ superphosphates and 50 kg ha⁻¹ potassium sulfate were applied according to recommendations of the soil testing laboratory. Plots were sown on Oct.11 2009 with a cone seeder at the rate of 70 plant m² and were 8 m in length and 2.4 m wide, with 8 rows of 0.3 m apart. Plots were plowed and disked after the winter wheat harvest in July. The plots were disked again before sowing in October. Weeds were controlled both mechanically and by Trifluralin (2.5 l ha⁻¹) as preplant and incorporated. Irrigation of each main plot was measured volumetrically by field calibrated gypsum block. The Ψ_{soil} at 30-cm depth was kept -0.025 MPa to maturity. The irrigation system was operated so that runoff did not occur. At maturity, 10 plants were taken randomly from each subplot for recording the following morphological, yield components and grain yield.

- Plant height (cm); from the cotyledonary node till the top of the plant.
- Specific seed weight (average weight of 1000 seeds in grams); recorded on 10 random samples from each sub-plot.

- Seed yield. Center six rows (of 8 rows) of each plot were harvested for grain yield and converted to grain yield per hectares.
- Harvest index. [wt. of grain/ (wt. of grain +straw)]
- The oil concentration of a sample of whole seeds from each plot was determined by Soxhlet methods [32].
- Oil yield (kg/ha); calculated by multiplying seed oil percentage ×seed yield per ha.

Samples were dried in a forced-air oven at 70°C for 48 h.

After determination of the oil yield (in the dry matter), fatty acids were esterified as methyl esters [32] and analyzed by Agilent 6890 N GC equipped with a DB-23 capillary column (60 m x 0.25 µm) and a FID (Flame Ionization Detector) detector. The carrier gas was helium, at a flow rate of 1.2 mL/min. Both injector and detector temperatures were kept at 250°C. Column temperature was initially kept at 165°C for 15 min and then increased to 200°C at a rate of 5°C/min, where it was maintained for 15 min. Samples of 1 µl were injected by auto-sampler, in the split mode (1:50). The fatty acid identification was performed by retention time comparisons with corresponding fatty acid methyl ester standards. The standards were purchased from Sigma-Aldrich Ltd.

Data were analyzed by analysis of variance [33]. When significant differences were found ($P=0.05$) among means, Duncan's multiple range test (DMRT) were applied.

RESULTS AND DISCUSSION

Plant Height: There was significant difference observed in gamma irradiation and interaction between cultivars and gamma irradiation in this trait (Table 2). The highest plant height was related to 100 and 200 Gy, resulting to lodging in some plots and the lowest plant height was belonged to 200 Gy (Table 3). Okapi cultivar and 200 Gy and Tallayeh cultivar and 500 Gy produced the highest and the lowest plant height, respectively (Table 4). The stimulating effect of low doses of gamma rays irradiation on plant growth may be due to stimulation of cell division or cell elongation, alteration of metabolic processes that affect synthesis of phytohormones or nucleic acids [34]. On the contrary, Banerji and Datta [35] and Shukla and Datta [36] found a reduction in plant height, branches and leaves number when root cuttings of *Chrysanthemum* were irradiated with low doses of gamma rays (1.5, 2 & 2.5 k-rad).

Table 2: Results of analysis of variance in cultivars and gamma irradiation

Sources	df	Plant height (cm)	1000-Kernel weight (g)	Grain Yield Kg ha ⁻¹	Harvest Index (%)	Oil percent	Linolenic acid (%)	Linoleic acid (%)	Oleic acid (%)
Replication	3	83.65	0.536	3257.36	945.34	47.34	3.735	0.605	0.27
Variety (V)	1	1532.6ns	43.32**	4085012.1**	298.3**	304.211ns	1.525ns	17.917**	165.1**
Error A	3	42.34	0.356	13177.06	15.52	31.076	1.08	0.876	1.605
Gamma Irradiation (GI)	5	4325.9**	0.0945**	1443219.7**	696.4**	278.31**	4.725**	54.715**	11.11**
C × GI	5	86.72**	0.067	6827.9**	78.24**	8.432**	0.52**	0.537**	0.195**
Error B	30	31.65	0.241	9733.317	11.948	7.652	0.67	0.351	0.095
CV (%)		14.35	19.41	8.31	13.25	7.31	12.18	21.22	18.71

*,** Significant at 0.05 and 0.01 probability levels, respectively. NS=non-significant at $P > 0.05$.

Table 3: Mean values of traits of canola cultivar at six gamma irradiations

Treatments	Plant height (cm)	1000-Kernel weight (g)	Grain Yield Kg ha ⁻¹	Harvest Index (%)	Oil percent	Linolenic acid (%)	Linoleic acid (%)	Oleic acid (%)
Cultivars Tallayeh								
Okapy	92.35A	4.34B	3689.25B	28.7B	45.7A	11.27A	31.12A	55.7B
Gamma Irradiation	90.12A	5.19A	3946.26A	33.4A	46.2A	11.01A	27.41B	60.24A
0								
100	93.24A	4.85B	3876.77A	31.2A	42.71B	11.01A	28.9B	62.37A
200	94.15A	5.26A	3985.26A	31.5A	48.32A	11.45A	28.12B	61.41A
300	94.01A	5.15A	3201.45B	30.3A	47.45A	11.1AB	33.19A	61.52A
400	85.28B	3.42C	3112.17B	25.7B	41.45B	10.6B	27.7B	62.11A
500	82.36B	3.35C	2436.12C	20.8C	37.22C	10.7B	26.9B	61.52A
	78.47C	2.89D	1844.19D	18.6D	36.19C	9.1C	22.1C	52.21B

Same letters in columns are not significantly different at $P \leq 0.05$.

Table 4: Mean values of interaction between cultivar and gamma radiation

Treatments	Plant height (cm)	1000-Kernel weight (g)	Grain Yield Kg ha ⁻¹	Harvest Index (%)	Oil percent	Linolenic acid (%)	Linoleic acid (%)	Oleic acid (%)
V1G11	93.3A	4.01B	3619.17B	29.6B	47.18AB	12.1AB	33.17A	59.31B
V1G12	93.3A	4.38B	3715.6AB	29.8B	49.17A	12.7A	34.22A	58.12B
V1G13	94.2A	4.21B	3611.17B	30.6B	49.65A	11.7B	34.15A	59.45B
V1G14	82.7B	3.12C	3437.19D	25.7C	40.17B	9.6CD	29.64B	48.32D
V1G15	74.1C	2.17D	1821.71F	19.6E	31.21C	8.7D	21.92D	47.32D
V1G16	65.6D	2.06D	1545.81G	17.7E	25.61D	7.6E	17.65E	35.17E
V2G11	94.4A	5.15A	3847.61A	32.6AB	47.61AB	12.4A	29.12B	63.19A
V2G12	94.5A	5.5A	3998.12A	32.7A	48.17A	12AB	28.98B	63.75A
V2G13	94.7A	5.65A	3895.25A	33.8A	47.12AB	12.7A	29.45B	60.31B
V2G14	79.6BC	3.82C	3012.26C	22.6D	32.17C	10.1C	24.17C	59.75B
V2G15	65.6D	3.65C	2136.17E	22.5D	25.17D	9.6CD	20.41D	52.31C
V2G16	59.8E	2.21D	1796.12F	18.6E	20.25E	8.5D	18.31E	47.31D

Same letters in columns are not significantly different at $P \leq 0.05$.

1000-Kernel Weight: Cultivar and gamma irradiation had significant effect on kernel weight at 1 % probability level (Table 2). The highest value of kernel weight was obtained in Okapi cultivar and 100 Gy gamma irradiation (Table 3). Significant planting date \times nitrogen fertilizer interactions were detected at $P \geq 0.01$ in this trait (Table 4). The stimulative effect of gamma rays irradiation on growth, especially at low doses was reported in several investigations. When Khan [7] exposed seeds of *Cicer arietinum* to gamma rays at doses ranging from 5 to 15 Krad, a stimulation of branching capacity, fresh weight and dry weight was reported. Similar results were observed by Kaul and Bradu [37] in *Atropa belladonna*, Suhas *et al.* [38] in *Cassia angustifolia*, Selenina and Stepanenko [8] in *Matricaria recutita* and Youssef *et al.* [9] in geranium

Grain Yield: Cultivar, gamma irradiation and interaction between them were significant in grain yield (Table 2). Among the two varieties, Okapi cultivar produced more grain yield than Tallayeh cultivar (Table 3). Grain yield more than 100 Gy gamma irradiation was reduced by 23 to 110 % (Table 3). Grain yield increased in response to applied gamma irradiation, with the grain yield of the crop that no received gamma irradiation being 5% more than control (Table 3). Among the five gamma irradiations and two canola cultivars, 200 Gy gamma irradiation and Talayeh cultivar produced the highest grain yield (Table 4). The presowing treatment with a magnetic field showed a positive impact on seeds of soybean, maize, peas, okra and beans leading to an increase of yield for soybean by 48%, for peas by 15%, for okra by 19% and for bean by 21%. High doses of gamma irradiation (over 15 k-rad) were reported to be harmful in several

studies. Ramachandran and Goud [39] reported that higher doses of gamma irradiation (40-120 k-rad) reduced plant height, number of leaves and branching capacity of safflower. A reduction in plant height, branches number, leaves number and size was found when root cuttings of *Chrysanthemum* were irradiated with 20 or 25 k-rad gamma rays [35].

Harvest Index: There was significant difference between cultivar and gamma irradiation and interaction between them in this trait (Table 2). 100 Gy gamma irradiation was more efficient than other treatments to increase harvest index (Table 3). Significant cultivar \times gamma irradiation was detected at $P \geq 0.01$ on harvest index. Okapi cultivar and 200 Gy gamma irradiation produced the highest value. Gunckel and Sparrow [40] supported our observations that gamma rays are known to influence plant growth and development by inducing cytological, genetically, biochemical, physiological and morphogenetic changes in cells and tissues. Several workers have studied effect of gamma rays on seed germination of Gymnosperms. The higher exposures were usually inhibitory [14-16]. The highest exposures are usually inhibitor on seed germination of Gymnosperm and Angiosperm [23] whereas lower exposures are sometimes stimulatory [41, 18].

Oil Percent: Gamma irradiation and interaction between cultivar and gamma irradiation were significant in oil percent (Table 2). 100 Gy gamma irradiation produced the highest oil seed content. Increasing in gamma irradiation more than 100 Gy decreased oil seed about 18% to 36% (Table 3). Among the five gamma irradiations and two canola cultivars, 100 Gy gamma irradiation and Okapi cultivar produced the highest grain yield (Table 4).

However it was in the same statistical group with Tallayeh and Okapi cultivar with 100 Gy gamma irradiation. Frank and Lendvi [42] and Mahmoud [11] reported an increase in carbohydrates and soluble sugars in response to seed irradiation. However, a decrease in carbohydrate content was reported in other studies [43,44]. Inoue *et al.* [45] demonstrated that irradiation of rice and maize seeds had no significant effect on soluble sugar content in the produced plants.

Our results are supported by previous published studies that report an increase in oil production by gamma irradiation in several plant species [9, 46, 47]. However, high dose of gamma irradiation decreases the oil production in other studies [12, 48, 49].

Fatty Acids: Table 3 and Table 4 show summary of the fatty acid composition and percentage of unsaturated fatty acids. The unsaturated fatty acid contents varied from 7.6 to 63.7 %. Generally, canola oil contains approximately 9-11% saturated (5-6% palmitic acid and 4-5% stearic acid) and 75-90% unsaturated fatty acids (50-55% linolenic acid, 15-20% oleic acid) [4]. The highest linolenic acid and linoleic acid obtained in 100 and 200 Gy gamma irradiation with average 11.45 % and 32.19%, respectively (Table 3). linolenic acid and linoleic acid decreased with increasing in gamma irradiation. Conversely, oleic acid content did not change with increasing in gamma irradiation, except in 500 Gy irradiation (Table 3). The best combination in order to obtain the highest linolenic acid and linoleic acid was 100 Gy gamma irradiation and Tallayeh cultivar (Table 4). The highest oleic acid was also obtained in Okapi cultivar and 100 Gy gamma irradiation (Table 4). The ionizing radiation might affect the quality of oils by increasing oxidation rate. It may also produce active species like free radicals, which initiate certain chemical reactions that might also result in the rancidity of oil and fats [50]. Irradiation of lipid induces the production of free radical, which react with oxygen, leading to the formation of carbonyls, responsible for the alteration in food's nutritional and sensorial characteristics [51]. Ionizing radiations effects the fatty acid composition of natural fats and the lipid peroxide formation as a result indicates that the peroxide value of fats and oils would increase with radiation [52].

A study on the effect of radiation with gamma rays of 0.5, 1.0 and 1.5 kGy, on the lipids present in different plant nuts revealed that the lipid extracted from the seeds have peroxide, anisidine and free fatty acid values higher than in their non-irradiated samples [53].

CONCLUSION

The results of this study demonstrate that 100 Gy irradiation was the best rate for increasing all traits. Higher doses of gamma irradiation decreased all traits in this study. Also, Okapi cultivar produced the highest values in all traits in this experiment. It recommends carry out the experiment under irrigation levels, soil types and other conditions.

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