Tropical Journal of Pharmaceutical Research December 2016; 15 (12): 2579-2585
ISSN: 1596-5996 (print); 1596-9827 (electronic)
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Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v15i12. 7

Original Research Article

Gamma radiation effects on crude oil yield of some soybean seeds: Functional properties and chemical composition of glycine max-ataem-7 seeds

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Received: 9 January 2016 *Revised accepted: 29 October 2016*

Abstract

Purpose: *To investigate the crude oil yield of eight different varieties of soybean (Glycine max L.) seeds after gamma radiation, and also to evaluate the antimicrobial activity and the chemical composition of G.max-Ataem7.*
Methods: *The seeds were irradiated with doses of 0 (control), 100, 200, 300, 400 and 500 Gy gamma radiation. Irradiation was performed in a cesium (Ce137) source. Extraction of the seeds was done with Soxhlet apparatus using petroleum ether. Antimicrobial activity was determined by the standard disc diffusion method. The chemical composition of the extracts was elucidated using gas chromatography-mass spectrometry (GS-MS)*
Results: *The highest crude oil yield was obtained at 300 Gy and and content of 35.09 % in Ataem7 seeds. There was a decrease in the total content of chlorophyll in Mutant1 (M1) plants after gamma radiation. However, the level of carotenoid increased in M1 plants. Extracts of the G.max-Ataem7 demonstrated strong antibacterial activity against S. aureus ATCC 25923 and E.coli ATCC 25922. The major components of G.max-Ataem7 were linoliec acid (C18:2n6) and oleic acid (C18:1n9) with a content of 60.31 and 21.64 %, respectively.*
Conclusion: *The results show that irradiation of is also can be improved if treated with appropriate doses of irradiation.*

Keywords: *Gamma Rays; Soybean, Glycine max-Ataem-7, Oil yield, Chemical composition*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Soybean (*Glycine max* L.) is a protein rich oil seed crop grown throughout the world. Soybean contains significant amounts of all the essential amino acids and fatty acids for humans, which are often used to prepare extracts or powders for medicinal use [1]. There is scientific attention on the health advantages of soybeans in both *in vivo* and *in vitro* experiments [2]. Therefore, plant breeding has gained importance especially soybean plants.

Radiation has been used effectively to cause suitable mutations for plant breeding improvement [3]. The percentage of spontaneous mutations is too low for breeding purposes in nature. Hence, chemical and physical mutagens can be used to encourage mutation [4]. Genetic variability is the most important requirement for effective crop development in terms of variant selection. Physical mutagens especially by using gamma-radiation, have been used by many researches for crop development [4].

Seed radiation is commonly used to implement a method to improve crop production, yield components and chemical composition. Some studies have investigated the effects of gamma radiation on soybean plants, including changes at the physicochemical levels. These effects include changes in the plant composition of chemical, crude oil yield and sensory [5].

The current investigation aims to explore scientifically the effects of gamma radiation on the crude oil yield of eight varieties of soybean seeds were studied, and the physiological effects, antimicrobial activity and chemical composition of the G.max-Ataem7 seed were investigated.

EXPERIMENTAL

Materials

The seeds were obtained from Izmir Aegean Agricultural Research Institute and the Antalya Western Mediterranean Agricultural Research Institute in 2011. The soybean seeds variety of Umut-2002, Ataem-7, Arısoy, Cinsoy, Nova, Üstün, Mitchell and Batem-Erensoy were used for the experiments. The irradiated and non-irradiated seeds were placed in sealed bags and were stored at room temperature (20 °C) without exposure to direct sunlight.

Methods

Gamma radiation application

The gamma radiation doses [0 (control), 100, 200, 300, 400 and 500 Gy] were applied to the soybean seeds. Irradiation was performed in a cesium (Ce 137) Gammacell 3000 Elan source with a dose rate ~9.75 Gy/min (2900 Ci) in the Department of the Radiology at the Pamukkale University Faculty of Medicine. Irradiated and non-irradiated samples were stored at room temperature (20 °C).

Determination of crude oil yield

After the application of gamma radiation, about 4 g of crushed seeds were extracted with Soxhlet apparatus (GFL, SR1050 Inc) with petroleum ether being used as a solvent. The extraction was carried on for 6 h with 250 mL of solvent. The extracts were concentrated, and the solvent was removed under reduced pressure at 40 °C using a rotary evaporator (IKA RV-10, Germany) [6].

Physiological effects of gamma radiation on M1 plant

Twenty seeds were placed in plastic vials. Seeds were sown in a mixture of pet/vermiculture (2:1). Vials were placed in a greenhouse at the Plant Genetics and Agricultural Biotechnology Application and Research Center (BIYOM) at 20-25 ºC standard conditions. Seedling and root length was measured using a measuring tape (cm). The seedling and roots fresh weight was measured with precision scales (g) (Precisa, XB 220A, Swiss). The seedling and roots fresh sample was dried in an oven at 70 ºC for 48 h [7]. Dry matter qualitative determination was made following the methods laid out in [8].

Photosynthetic pigment analysis (fresh weight)

Photosynthetic pigments were determined using the Arnon method [7]. Chlorophyll was extracted from a similar position with regard to the leaves of ten plantlets. The optical density (OD) was measured using with a UV-VIS spectrophotometer (Optimum One Chebios, Italy) at 663 nm for chlorophyll-a, at 645 nm for chlorophyll-b and at 470 nm for carotenoid. The chlorophyll-a (Eq 1), chlorophyll-b (Eq 2), total chlorophyll (Eq 3) and carotenoid (Eq 4) concentrations were determined from the following equations [9].

Chl.a (mg/g) = (12.7 x OD663 - 2.69 x OD645)V/(1000 W) ………………… (1)

Chl.b (mg/g) = (22.9 x OD645 – 4.68 x OD663)V/(1000 W) ………………… (2)

Total Chl. (mg/g) = (20.2 x OD645 + 8.02 x OD663)V/(1000 W) ………………… (3)

Car. (mg/mL) = [4,07 x OD450-(0,0435 x kla+0,367 x klb)] …………………… (4)

where V is the total volume of acetone extract (ml) and W is the fresh weight (g) of the sample.

Antimicrobial activity of G.max-Ataem7 extracts

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were obtained from the American Type Culture Collection (ATCC) and *Micrococcus luteus* NRRL B4375 was obtained from the Northern Regional Research Laboratory (NRRL). The bacteria were cultured in Nutrient Broth (NB) (Difco) and were then incubated at 37 ± 0.1 ^{o}C for 24-48 h. The antimicrobial activity of the extracts were assayed using the standard disc diffusion method [10]. Inhibition zone was measured using a measuring tape (mm). Discs of petroleum ether (Merck) were used as negative controls. Discs of ampicillin (10 µg, Oxoid), Penicillin (10 U, Oxoid) and Gentamycin (10 µg, Oxoid) were used as positive controls.

Gas chromatography-mass spectrometry (GC-MS)

The seed oil was analyzed using GS-MS. The GS-MS analyses were carried out on an Agilent 7890 A GS-MS equipped with a HP-88 silica column (100m x 0.250 mm, film thickness 0.20 µm); the oven temperature was held at 60 ºC for 1 min and was then programmed to 175 ºC at 13 ºC and programmed their temperature to 215 ºC at 4 ºC/min [11]. The percentage oil composition of the extracts were determined using the MSDChem computer programme.

Statistical analysis

The experiments were carried out in triplicate and three measurements were conducted for each replicate. The data were analyzed by analysis of variance (ANOVA) using SAS software (Inc. Chicago, IL, USA, 1988). Statistical analysis was carried out using SAS computer software. Significant differences between values were determined using Duncan's Multiple Range test. $P < 0.05$ was considered statistically significant.

RESULTS

The effect of gamma radiation on the crude oil yield of eight different varieties of soybean (*Glycine max* L.) seeds is shown in Figure 1. The crude oil yields of *G. max* seeds were determined to be between 13.11 and 35.09 % for Batem-Erensoy and Ataem-7, respectively. The highest crude oil yield was obtained at 300 Gy dose in Ataem-7 seeds.

The physiological effects of *G. max*-Ataem7 (the variety which showed the highest oil yield) were studied in terms of all irradiation doses. This study has shown that gamma radiation exerts a significant inhibitory influence on the growth of M1 generation plants (Table 1). The highest plant height was related to control plants and a dose of 100 Gy, while the lowest plant height was related to a dose of 500 Gy.

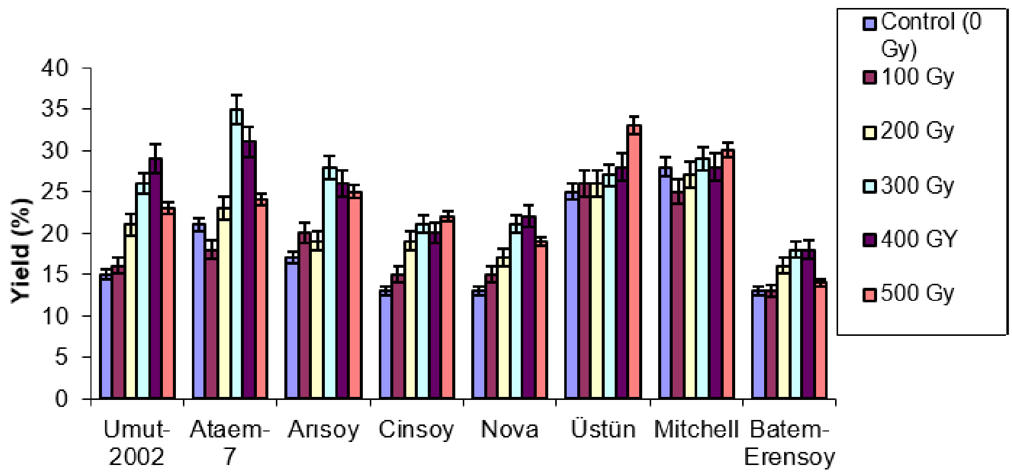

Figure 1: Effects of gamma radiation on crude oil yield of *Glycine max* seeds (dry matter, %)

Table 1: Growth properties of *G. max*–Ataem7 M1 generation

Variable	Seedling length (mm)	Root length (mm)	Number of leaves (no/plant)	Seedling fresh weight (g)	Seedling dry weight (g)	Rooth fresh weight (g)	Rooth dry weight (g)
Control	160	3	9	4.61	4,10	1,39	1,15
100 Gy	120	5	7	4,01	3,66	1,24	0,95
200 Gy	60^a	4	3	2,89	2,09	1,03^b	0,61^b
300 Gy	60^a	3	2	2,52	1,94	0,56^a	0,43^b
400 Gy	50	2^a	2	2,13^b	1,68^a	0,28	0,10
500 Gy	40	1^b	2	2,10^b	1,53^b	0,11	0,04

Significant at $p < 0.05$ and 0.01, respectively; NS: not significant at $p < 0.05$

The effects of gamma radiation on the chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid pigments of *G. max* Ataem-7 are shown in Figure 2. When compared to the controls, decreases in the total amounts of chlorophyll was detected in M1 plants. On the other hand, carotenoid amounts were increased in *G. max* Ataem-7 M1 plants.

The antimicrobial activity of *G. max*-Ataem7 extracts were determined against two Gram-positive [Gr(+)] and two Gram-negative [Gr(-)] bacteria (Table 2). All doses extracts of the *G. max*-Ataem7 demonstrated antibacterial activities against the *S. aureus* ATCC 25923, *E.coli* ATCC 25922 and *M. luteus* NRRL B-4375.

Table 3 provides a summary of fatty acid composition and the percentage of saturated and unsaturated fatty acids. Fifteen components of the extracts were obtained by soxhlet extraction and these were detected using GC-MS analytical methods. It was determined that, linoliec acid (C18:2n6) and oleic acid (C18:1n9) were the major components, with an average of 60.31 and 21.64 %, respectively.

DISCUSSION

The effects of gamma radiation on some of the properties of many plants and seeds have been investigated by researchers [12-14]. In this study, the crude oil yield of all extracts of *G. max* were positively affected by gamma rays.

Our results are supported by previous published studies that report an increase in oil production subsequent to gamma irradiation in several plant species [15]. However, Dixit *et al* [13] determined that the nutritional features i.e. fatty acids, protein and the oil of soybean seeds, remained stable after gamma radiation.

It has been noticed that environmental conditions can affect seed composition in terms of such features as oil and protein content.

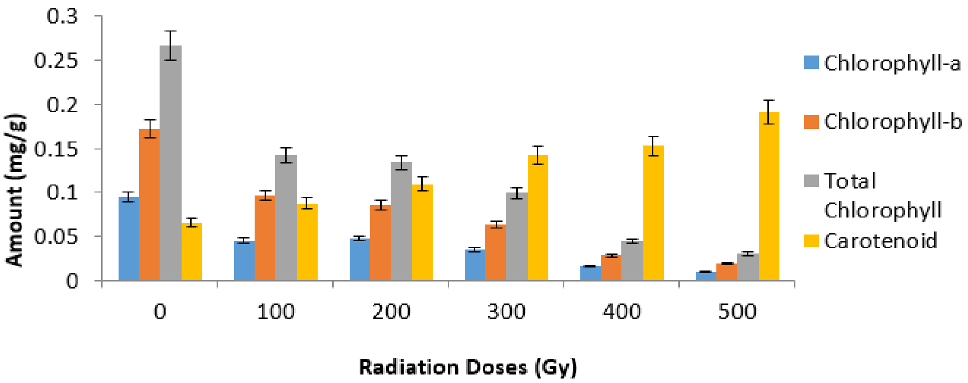

Figure 2: Effect of gamma radiation on photosynthetic pigments of *G. max*-Ataem7 M1 generation

Table 2: Antimicrobial activity of *G. max*-Ataem7 extracts

Extract	Concentration (µl/disc)	Inhibition zone diameter (mm) – Microorganism – Gr(+) *M. luteus* NRRL B-4375	Gr(+) *S. aureus* ATCC 25923	Gr(-) *E. coli* ATCC 25922	Gr(-) *P. aeruginosa* ATCC 27853
C	10µl	8 ± 0.5	9 ± 0.5	7 ± 0.5	-
E1	10µl	9 ± 0.5	10 ± 0.5	7 ± 1.5	-
E2	10µl	9 ± 1	11 ± 1	8 ± 0.5	-
E3	10µl	10 ± 1.5	12 ± 1.5	8 ± 1	-
E4	10µl	11 ± 0.5	13 ± 1	8 ± 1.5	-
E5	10µl	11 ± 1	14 ± 1.5	9 ± 0.5	-
Reference antibiotics					
A	10 µg	28	NT	21	NT
P	10 U	29	30	18	NT
G	10µg	NT	NT	NT	15

C: Control extract (not irradiated), E1: Applied 100 Gy dose extract, E2: Applied 200 Gy dose extract, E3: Applied 300 Gy dose extract, E4: Applied 400 Gy dose extract, E5: Applied 500 Gy dose extract, NT: Not tested, A: Ampicillin (10 µg), P: Penicillin (10 U), G: Gentamicin (10 µg), (–): No inhibition zone

Table 3: Chemical compositions of *G.max*-Ataem7 extracts in GC-MS methods

		Compound	Structure	Rt	C (%)	E1 (%)	E2 (%)	E3 (%)	E4 (%)	E5 (%)
Saturated fatty acids	1	Lauric acid ME*	(C12:0)	27,3	-	-	-	-	-	-
	2	Myristic Acid ME*	(C14:0)	30,4	0,36	0,35	0,12	0,24	0,23	0,21
	3	Palmitic acid ME*	(C16:0)	34,5	14,12	14,13	13,63	15,48	15,21	15,26
	4	Heptadecanoic (Margaric) acid ME*	(C17:0)	36,5	0,09	0,09	0,14	0,02	0,02	0,01
	5	Stearic Acid ME*	(C18:0)	39,1	3,82	3,71	3,14	3,31	3,28	3,51
	6	Arachidic Acid ME*	(C20:0)	43,3	0,45	0,33	0,29	0,11	0,11	0,09
	7	Pentadecanoic (Pentadecyclic) Acid ME*	(C15:0)	32,3	-	-	-	-	-	-
Unsaturated fatty acids	8	Myristoleic Acid ME*	(C14:1)	31,8	-	-	-	-	-	-
	9	Palmitoleic acid ME*	(C16:1)	35,5	0,14	0,13	0,07	0,33	0,21	0,19
	10	Oleic acid ME*	(C18:1n9)	40,3	21,03	21,02	15,48	21,64	21,27	21,14
	11	cis-11eicosenoic acid ME*	(C20:1)	44,9	-	-	-	-	-	-
	12	Linoliec Acid ME*	(C18:2n6)	42,1	54,78	54,80	60,31	53,38	53,34	53,72
	13	g-linolenic acid ME*	(C18:3n6)	44,2	4,08	4,63	6,03	4,75	4,71	4,03
	14	cis 11,14-eicosadienoic acid ME*	(C20:2)	48,3	0,20	0,21	0,31	0,38	0,33	0,35
	15	EPA (Eicosapentaenoic acid) ME*	(C20:5n3)	53,3	0.74	0,63	0,42	0.10	0,10	0,10

ME: Methyl Ester, Rt: Retention time, C (%): Control extract (not irradiated), E1 (%): Applied 100 Gy dose extract, E2 (%): Applied 200 Gy dose extract, E3 (%): Applied 300 Gy dose extract, E4 (%): Applied 400 Gy dose extract, E5 (%): Applied 500 Gy dose extract

Major differences in the protein content of the soybean species in different climatic conditions have been observed [16]. However, the information on the chemical composition and antimicrobial activity of soybean seeds exposed to gamma radiation was limited. In general, seedling length of M1 plants was decreased by gamma radiation while increase in the dose of gamma rays. The stimulating affect of low doses of gamma radiation on plant development may be due to the stimulation of cell elongation or cell division involving the modification of metabolic processes that affect the synthesis of nucleic acids [12].

On the other hand, one study has reported that there is a reduction in the number of plant leaves and branches of plants irradiated with low doses of gamma rays [17]. In our study, it has been found that gamma rays have a negative effect on root and leaf growth. Similarly, Alikamanoğlu *et al* [14] found that leaf number of *Pawlonia tomentosa* decreased according to the applied dose (10 and 25 Gy) and this physiological damage in M1 generation caused by radiation.

Aparna *et al* [18] also showed that higher doses of gamma radiation have a negative effect on plant growth. Similarly, there are some reports which indicate that higher doses of gamma radiation are usually inhibitory [4], whereas lower doses are sometimes stimulatory [16].

Afify and Shousha [19] reported that gamma radiation caused molecular changes. Hence, it is presumed that molecular compositions caused changes in secondary structure, especially at higher dose levels.

The antimicrobial activity of extracts has been evaluated *in vitro* against four bacterial species (Table 2). Compared to reference antibiotics, extracts of the *G. max*-Ataem7 showed antimicrobial activities particularly against Gr(+) bacteria. However, all of extracts showed no antimicrobial activities against *P. aeruginosa* ATCC 27853. The antimicrobial activity of the soybean plants were reported [20,21]. Similarly, the methanol extracts of *G. max* showed antibacterial activity against *S. aureus* [20,21]. On the other hand, methanol extracts of *G. max* showed no antibacterial activity against *P. aeruginosa* [20] and *P. fluorescens* [21].

It was determined that, linoleic acid, oleic acid and palmitic acid were the major components with an average of 60.31, 21.64 and 15.48 %, respectively. Azcan *et al* [22] obtained results for the oil content of thirty soybean seed varieties, and determined that the average percentage of linoleic and oleic acid content in the Ataem7 was 51.25 % and 27.34 %, respectively. The highest linoleic acid percentage was observed in 200 Gy irradiated seed (60.31 %) as compared to a control (54.78 %). Palmitoleic acid (C16:1) and cis 11-14 eicosadienoic acid (C20:2) were shown to increase fatty acid percentage after being dosed with gamma radiation.

Ionizing radiation might affect the quality of oils by increasing the degree of oxidation. It may also present active molecules such as free radicals, which may start chemical reactions that might also result in the souring of oil and fats. The radiation of lipid stimulates the production of free radicals which react with oxygen, and are responsible for the formation of carbonyl groups and the modification sensorial and nutritional features of foods.

Ionizing radiations affect the fatty acid composition of natural oils, and the lipid peroxide formation as a result indicates that the peroxide value of oils and fats would increase with irradiation [23]. A study of the effect of radiation involving gamma radiation (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 non-irradiated samples [15].

CONCLUSION

The results of this study demonstrate that gamma radiation has a generally positive effect on the crude oil yield of *G. max* seeds. The results showed that the highest crude oil yield (35.09 %) was obtained from a dose of 300 Gy applied to Ataem-7 soybean seeds. Similarly, the antibacterial activity of *G. max*-Ataem7 extracts showed high activity in terms of Gr(+) bacteria, especially *S. aureus* ATCC 25923. Our results may suggest that gamma radiation application of G.max-Ataem7 seeds was effective in substantially improving oil yield and antimicrobial activity.

DECLARATIONS

Acknowledgement

This study was supported by The Scientific Research Projects Coordination Department of Pamukkale University, Project No: 2012FBE013. Thanks to the Pamukkale University Plant Genetics and Agricultural Biotechnology Application and Research Center (PAU BIYOM).

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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