Effect of Gamma Irradiation on Some Biochemical Parameters and Physico-chemical Properties of Oil in Soybean [*Glycine max* (L.) Merr.]

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Abstract

The soybean (*Glycine max* l. merr) is one of oil seed plants from leguminous family. In this study, the effect of gamma radiation on chlorophyll, soluble and insoluble carbohydrate and composition oil seeds were investigated in soybean. Soybean variety J.K was irradiated with 0,100,200 and 300 gamma ray. The treatment and control seeds planed in with four replicates. The highest amount of total chlorophyll content was obtained in control plants. The highest amount of soluble carbohydrate was obtained in 200 gamma ray treatments. The contents of carbohydrates in the leaves tend to increase with increasing gamma ray doses. The elevated levels of the total soluble and insoluble carbohydrates in leaf are considered to be playing an important role in the osmotic adjustment. The results showed that the highest amount of total protein content was obtained in control plants. The contents of protein in the leaves tended to decrease with increasing gamma ray doses. The oil soybean seeds were extracted and analyzed for their chemical and physical properties such as acid value, percentage free fatty acids (% FFA), iodine value, and refractive index.

Key words: Gamma Irradiation; Oil; Chlorophyll; Carbohydrate; Protein; Soybean

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Introduction

Soybean seed is a major source of high-quality protein and oil for human consumption (Katerji et al., 2001). Approximately 18% to 21% of soybean seed dry weight is oil in the form of triacylglycerol. From 24 to 40 days after flowering, oil percentage increases rapidly and by the end of this period accounts approximately 30% of the total oil of the mature seed. The remaining 70% is synthesized during the next 40 to 64 days after flowering; also a period of seed desiccation (Hajduch et al., 2005). Growth; development and yield of soybean are the results of genetic potential interacting with environment. Minimizing environmental stress will optimize seed yield (Mc Williams et al., 2004). Research on the basic interaction of radiation with biological systems has contributed to human society through applications in medicine, agriculture, pharmaceutical uses, and other

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technological developments. In agricultural science and food technologamma ray, recent research has elucidated new potential applications for radiation for example, high doses of ionizing radiation have been shown to inhibit growth of microbial infestations in seeds. The application of mutation techniques i.e., gamma rays and other physical and chemical mutagens has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies (Hajos., 2009). Gamma rays are known to influence plant growth and development by inducing cytological, genetically, biochemical (Hameed et al., 2008), physiological and morphogenetic changes in cells and tissue (Gunckel & Sparrow, 1961).There are also many reports supporting the use of gamma irradiation as a fungicidal agent ([Aziz et al., 2007] and [Maity et al., 2004]). However, the viability, and sometimes the developmental process, of the seedling or the plant have been seriously hampered by radiation (A.P. Casarett, Radiation biology, Prentice-Hall lnc, New York (1996) pp. 284–312.Casarett, 1996). Gamma radiation is also used to increase genetic variation in plants and to render them more productive and resistant. Many plant varieties with commercial and agricultural importance have been developed using gamma radiation (Mial et al., 1966 and Shah et al., 1994). Assessing the negative effect of irradiation after exposure, as well as determining exposure conditions, radiation dose-response curve, and mutation dosages, have critical importance. Among other parameters, the level and oil may change due to radiation. The aims of study evaluate the effects of gamma irradiation on chlorophyll , carbohydrate, protein and composition oil seeds of *Glycine max* L. merr Var.JK during gamma irradiation.

Materials and Methods

Seeds of soybean (*Glycine max* L. merr) were collected from central investigation of cultivation in sari, Iran. Fully mature and perfect seed were irradiated with 3 doses of gamma radiation (0,100,200 and 300 Gamma ray) by a 60 Co source. After irradiation, seeds were sown in research field of central investigation of cultivation in Joybar, along with untreated controls in four replicates with completely randomized design. Soybean seeds control and treatment planted 7.6 cm apart within each row. Rows were 46 cm apart to provide a population of 300.000 plants ha^{-1}.

Each experimental plot consisted of three adjacent rows 2.4m in length. The field plot design included 16 plots (four replications with four treatment plots).

Measurement of chlorophylls

The content of chlorophyll was measured by Arnon method (Arnone, 1994). Chlorophyll was extracted in 80% (V/V) aqueous acetone and absorption measured in spectrophotometer at 645 and 663 nm.

Measurement of carbohydrates

Soluble and insoluble carbohydrates were determined by the method of Fales (1951). Fresh weight (100 mg) of the leaf tissue from each sample was used using the youngest fully expanded leaf.

Estimation of total protein

The "Lowery method" (Lowry 1951) was used for total protein determination of gamma treated and untreated control seeds.0.5 g of each seed type was extracted with 10 ml of 0.2 M phosphate buffer (pH 7.4), the amount of protein was determined by comparing to an authentic BSA standard (Sigma, USA) and expressed in terms of percentage. All chemicals were procured from Sigma st. louis, USA. The seed kernels were ground using a mechanical grinder, and defatted in a soxhlet extractor apparatus, using hexane (boiling point of 40–60 °C). The extracted lipid was obtained by filtrating the solvent lipid contained to get rid of the solid from solvent before the hexane was removed using rotary evaporator apparatus at 40 °C. Extracted seed oil was stored in freezer at 2 ˚C for subsequent physicochemical analysis.

Physico-chemical analysis of oil seed

Oil Content

The weight of oil extracted from 10 g of seeds powders was measured to determine the lipid content. Result was expressed as the percentage of oil in the dry matter of seed powders.

Acid value, % FFA

Acid value of seed oil was determined according to AOAC Official Method Cd 3a- 63. Percentage free fatty acids (FFAs) were calculated using oleic acid as a factor.

Iodine value

Iodine value of seed oil was determined according to AOAC Official Method 993.20.

Refractive index

Refractive indexes were measured by AOCS Official methods Tp 1a-64 (AOCS, 1989).

Statistical Analysis

The experimental design was a completely randomized factorial. Analysis of variance (ANOVA) for all the measured Variables was performed by MstatC. the treatment means were separated using Duncan's multiple range test (DMRT) taking P<0.05 as Significant.

Results

The chlorophyll (a,b and total) content is of particular significance to precision in agriculture as an indicator of photosynthetic activity. Chlorophyll contents of these gamma irradiation when compared with control showed that total chlorophyll was increased with the increase in radiation dose. Chlorophyll content of seeds in both genotypes was affected by gamma radiation. Under gamma irradiation, a general reduction in chlorophyll b, content was observed comparison among different doses of gamma radiation(Fig.III).The results showed that seedlings were exposed to gamma irradiation (200 and 300 gamma ray) exhibited an decrease in chlorophyll *a, b* and total chlorophyll levels (Fig. I,II,III), when compared to non-irradiated treatment. As illustrated in Fig. I, the concentration of chlorophyll *a* was higher than

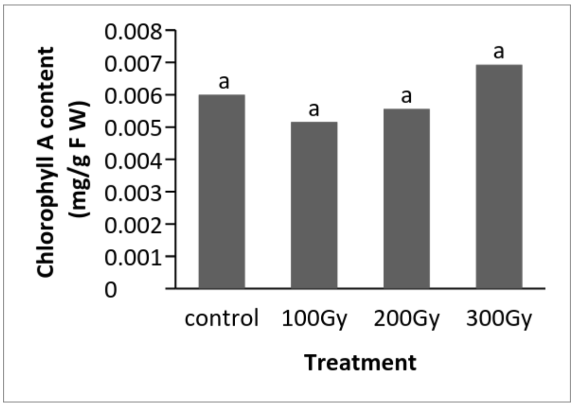

Fig. I. Effect of gamma ray on chlorophyll a content (mg/g FW) of soybean C.v:J.K. Means with different letters are significantly different (p<0.05).

chlorophyll *b* in both seedling groups (irradiated and non-irradiated treatment). Seedlings exposed to gamma irradiation no significant changes in chlorophyll *a, b* and total chlorophyll contents. In contrast, seedlings irradiated at gamma showed a decrease of chlorophyll *a, b* and total chlorophyll, as compared to the non- irradiated seedlings. The lowest total chlorophyll content was obtained in seedlings irradiated at 100 gamma ray.

The contents of soluble and insoluble carbohydrates in the leaves of the treated seedlings of soybean plants are shown in Fig. IV and V. It can be seen that the contents of carbohydrates (soluble and insoluble) in the leaves tends to increase with increasing gamma irradiation (Fig. IV and V.). It is apparent from the results that the soluble carbohydrate content in

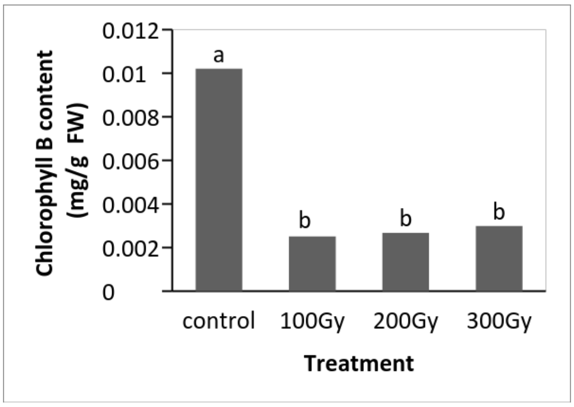

Fig. II. Effect of gamma ray on chlorophyll b (mg/g FW) content of soybean C.v:J.K. Means with different letters are significantly different (p<0.05) the leaves was higher in 200 Gamma ray treatment plants compared with control (Fig. IV).

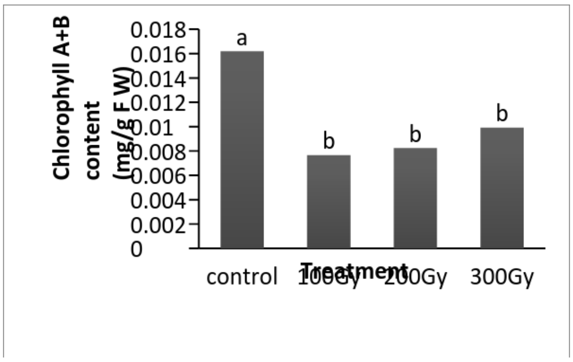

Fig. III. Effect of gamma ray on chlorophyll a+b (mg/g FW) content of soybean C.v:J.K .Means with different letters are significantly different (p<0.05)

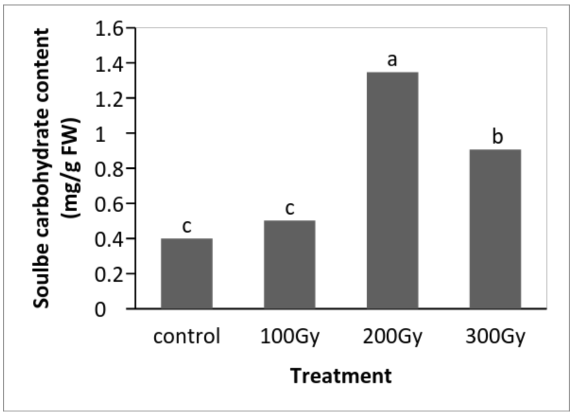

Fig. IV. Soluble carbohydrate content (mg/g FW) of leaves soybean C.v:J.K with gamma irradiation. Means with different letters are significantly different (p<0.05)

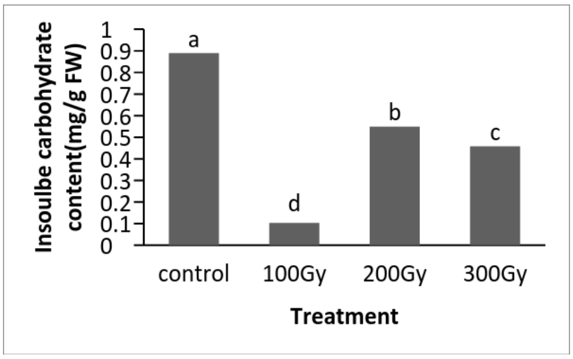

Fig. V. Insoluble carbohydrate content (mg/g FW) of leaves soybean C.v:J.K with gamma irradiation. Means with different letters are significantly different (p<0.05)

In contrast, the insoluble carbohydrate in the leaves of 100 gamma ray treatment was much lower than in the other treatment and control plants(Fig. V)

Biochemical differentiation based on protein content revealed that seedlings irradiated at 100, 200 and 300 Gamma ray. Data analysis shown (the $p<0.05$) significant difference between gamma radiation in protein content in control and treatment plants. The protein contents were decreased after imposing different levels of gamma radiation as compared with non-irradiated(control) plants (Fig. VI). However, protein contents were decreased with increasing irradiation. The lowest protein content observed in 300 Gamma ray as compared to control plants. Maximum content of protein (mg/g fresh weight) was observed in control plants. The protein content in leaves soybean decreased in the all treatment gamma irradiation plants (Fig. VI).

Physico-chemical Properties

The oil content of *soybean* oil seeds was decreased by gamma irradiations. The determined at 17.525%. Oil content in control plants and 100 Gamma ray treatments were found higher than 200 and 300 gamma ray treatment plants(Fig. VII)

The iodine value of soybean oil in control plants was determined at 123.025, and the iodine value in 200 Gamma ray treatment plants were 133.075. The lowest Iodine value was observed in control plants as compared to maximum Iodine value were observed in 200 Gamma ray treatment plants (Fig. VIII).

Experimental result showed that highest oil seeds have FFA content 0.96% in 200 Gamma ray treatment plants and the lowest oil seeds have FFA content 0.061% in 100 Gamma ray. The high FFA content (>1% w/w) will happen soap formation and the separation of products will be exceedingly difficult (Fig.IX).

The moisture contents have significant effects on the transesterification of glycerides with alcohol using catalyst (Goodrum, 2002). The

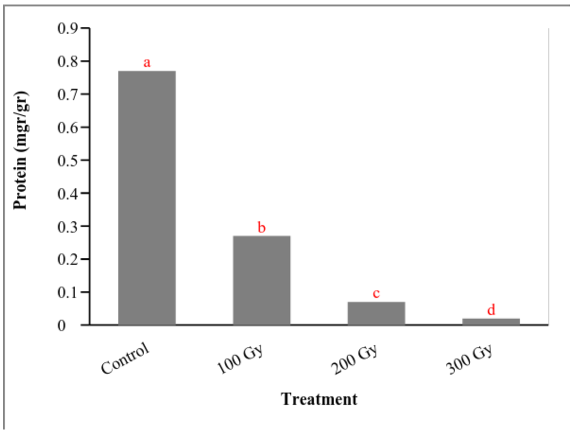

Fig. VI. Effect of gamma irradiation on protein content (mg/g FW).Gamma-raydecreased in protein content in leaves. Data analysis shows that between Gamma-ray and control plants on the amount of protein (mgr/gr FW) significant difference p <0.05 there.

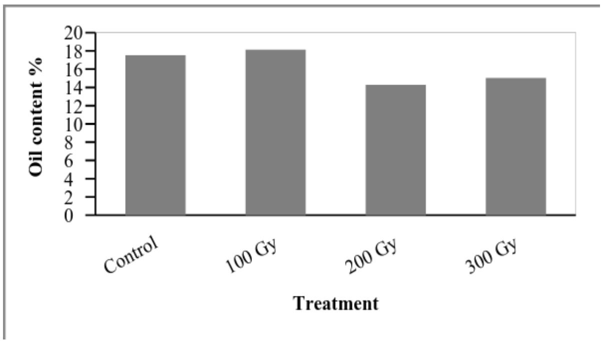

Fig. VII. Data analysis shows that between Gamma-ray and control plants on the oil content % significant difference p <0.05 there.

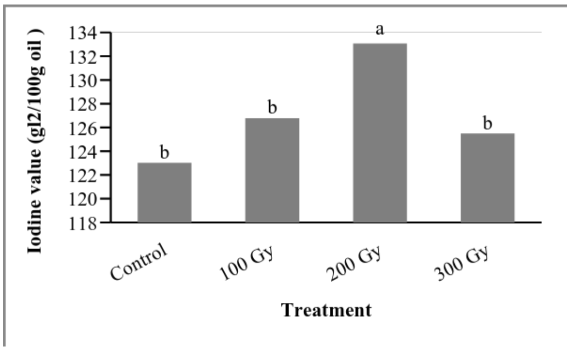

Fig. IX.Data analysis shown that between gamma radiation in Iodine value in control and treatment plants (the p <0.05) significant difference.

highest moisture contents were observed in 200 Gamma ray (18.075%) and lowest moisture contents in 100 Gamma ray (16%) (Fig.X).

The refractive index analysis shows only 200 Gamma ray significant different to other treatment, with the values between 14.667 in 200Gamma ray and 14.655 in control plants (Fig. XI).

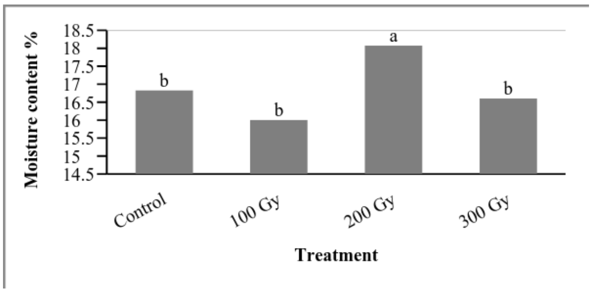

Fig.X . The gamma irradiation moisture content%. Gamma-ray increased in moisture. Content percent in oil seeds. Data analysis shows that between Gamma-ray and control plants on the amount of moisture percent significant different in p <0.05.

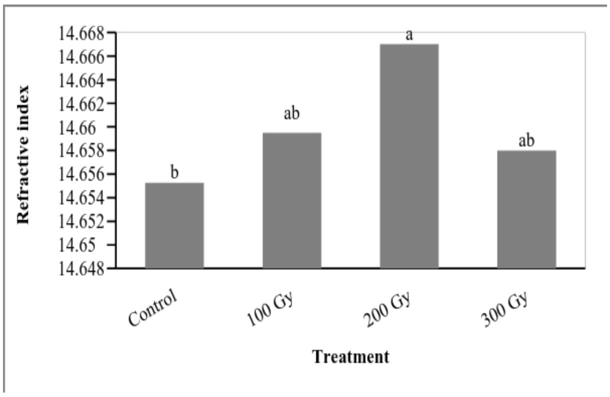

Fig. XI. Effect of gammairradiation on refractive index in soybean oil seeds. Gamma-ray increased in refractive index. Data analysis shows that between Gamma-ray and control plants on the amount of refractive index significant different in p <0.05.

Discussion

Gamma radiation induces various physiological and biochemical alteration in plants. The irradiation of plants with high dose of gamma rays disturbs the hormone balance, leaf gas-exchange, water exchange and enzyme activity (Kiong et al., 2008). These effects include changes in the plant cellular structure and metabolism such as dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidant system, and accumulation of phenolic compounds. Based on transmission electron microscope observations, chloroplasts were extremely sensitive to gamma radiation compared to other cell organelles, particularly thylakoids being heavily swollen (Wi et al., 2007). In this study, the chlorophyll content of gamma irradiated soybean displayed a gradual decrease at 200 Gamma ray dose. In addition, it can be observed that the concentration of chlorophyll a was relatively higher than chlorophyll b in irradiated and non-irradiated plants. Kiong et al., (2008) reported that the reduction in chlorophyll b is due to a more selective destruction of chlorophyll b biosynthesis or degradation of chlorophyll b precursors. Furthermore, Kim et al., (2004) have evaluated the chlorophyll content on irradiated red paper plants; their results showed that plants exposed at 16 Gamma ray may have some significant increase in their chlorophyll content that can be correlated with stimulated growth. These results are in agreement with the findings of Golampour et al., (2010). This is strong evidence that photosynthesis is the main source of accumulating organic solutes under water stress. Meyer & Boyer (1981) showed that cutting the photosynthetic cotyledons from Soybean seedlings prevented solute accumulation and osmotic adjustment as also concluded by Kutachera & Kohler (1994). The accumulation of organic solutes (soluble and insoluble carbohydrates) might play an important role in increasing the internal osmotic pressure (Zidan & Al- Zahrani, 1994). Many plants, which are stressed, accumulated starch and soluble carbohydrates (Green way & Munns, 1980; Rathert, 1984). This accumulation has been attributed to impaired carbohydrate utilization (Munns & Jermaat, 1986).This is consistent with a blockage in utilization of sugars in the growing tissues and a subsequent build-up in the rest of the plant. A reduction in photosynthesis could be due to feedback inhibition by the high sugar concentrations in the mesophyll cells. It is appearing in the beginning of growth that soybean seedlings are not deficient in carbohydrates and that the supply of carbon compounds is not limiting their growth. So, after prolonged periods of exposure to gamma irradiation the levels of reserve carbohydrates increased, particularly in the leaves. The protein and oil quality effects of gamma radiation were studied on JK soybean Varity, which has high economical and nutritional value. Measuring of protein is one of the best parameters to study plant response against radiation (Oldacay 2002 and Wada et al., 1998). Different studies showed that protein contents change in plants under stress condition (Schmidet al., 1985). In this study, the protein content of JK soybean decreased after gamma radiation. A significant decrease of 80% was observed in the group that was exposed to 300 Gamma ray. These results are in disagreement with the findings of Alikamanoglu et al., (2011) on soybean who noticed that treating seeds with high rates of gamma the soluble protein content increased. The decrease oil content in gamma irradiation plants indicated that gamma irradiation on soybean oil seeds are suitable as edible vegetable oil (Azam et al., 2005). The iodine value is a measured of the unsaturation of fats and oils. Higher iodine value indicated higher unsaturation of fats and oils (Knothe, 2002; Kyriakidis and Katsiloulis, 2000). The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides. High iodine value of 200 Gamma ray treatments are caused by high content of unsaturation fatty acid such as oleic acid and linoleic acid. The high iodine value in 200Gamma ray treatment stability shows that the seed oil upholds the good qualities of semidrying oil purposes (Eromosele et al., 1997).The FFA and moisture contents have significant effects on the transesterification of glycerides with alcohol using catalyst (Goodrum, 2002).The results of this research showed that different doses of gamma radiation had different effects on biochemical characteristics, such as increasing of protein content and chemical and physical properties of oil seeds such as increasing of iodine value, free fatty acid, moisture content and refractive index but, the oil content decreased in all gamma irradiation plants. It is clear that this technique can be used for production of a mutant with ability for environmental stress tolerance.

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