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EFFECT OF GAMMA IRRADIATION ON GERMINATION AND BIOCHEMICAL CONSTITUENTS OF SOYBEAN LEAVES

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ABSTRACT

The present work was conducted to study the effect of gamma irradiation ^{60}Co of a wide range of doses from 5-100 Gy for soybean seeds on the germination, chlorophyll a and b as well as carotenoids. Also, changes in biochemical contents of carbohydrates, DNA and RNA and proteins of soybean leaves were investigated. The results obtained cleared that, the germination percentage of soybean seeds increased up to 100% with respective dose of 40 Gy. Also, the maximum chlorophyll a, b and carotenoid contents were increased at the same dose. Besides, activation in carbohydrates, ribonucleic acids and proteins were detected.
Keywords: Gamma Irradiation / Germination / soybean leaves.

INTRODUCTION

In the last decades, gamma irradiation has been drawn the attention as a new and rapid method to improve the qualitative and quantitative characters of many crops. Several studies were carried out on the effect of ionizing radiation on plant growth following irradiation of seeds Gunckel and Sparrow (1981). An increase in seed germination, faster vegetative growth, early flowering and increased yields were the common results. Irradiation of seeds with suitable dose of gamma rays produces physiological changes in the plant tissues that in turn may be affecting the yield of plant. (Aleksander *et al*., 1986; Ragab 1994) showed that, stimulation was not observed immediately after seed germination at later development stages with low doses. While, high doses cause failure of germination and plant growth. Also, irradiation of seeds with low doses stimulated the production of chlorophyll, whereas higher doses caused chlorophyll deficiency. (Singh 1971; Farag *et al.,*1986) found that, some varieties of soybean seeds grown in Egypt have crude protein ranged from 35.88 – 39.31%, whereas, oil content ranged from 18.26 – 22.35%.

The properties of soybean protein following gamma irradiation at dose range from 5-20 KGy was investigated by Myung-Woo and Il- Jun (1994). The results obtained cleared that, irradiation doses above 10 KGy caused a decrease in 75 and 115 components and an increase in 25 and 155 components. The effect of gamma radiation with doses of 2.5, 5.0 and 8.0 KGy on soybean seeds was studied by Yonies (1997). The results showed significant change in the chemical constituents of proteins, carbohydrates and lipids. Irradiation of soybean seeds with high doses of gamma source caused failure in germination, plant growth, root growth and yield; while lower doses stimulate them (Olfat *et al.,* 1985). Exposed seeds

of pea (*Pisum Sativuml*.) to different doses of gamma irradiation i.e. 0, 0.5, 2.5, 5, 10, 15, 20, 25, 30, 35 and 40 Krad to study its effects on seed germination, growth biochemical aspects and yield (Selim and El-Banna., 2001). The authors showed that doses of 0.5, 2.5, 5 and 10 Krad caused a significant increase in the germination percentage while doses of 15, 20, 25, 30, 35 and 40 Krad decreased it. The effect of gamma irradiation on dry bean was investigated by Celik *et al* (2001).

The aim of the present study was to evaluate the effects of gamma irradiation with different doses on germination and biochemical constituents of soybean leaves.

MATERIALS AND METHODS

Materials

Source of grains and seeds:

The used soybean seeds (*Glycine max* L.) Cultivar Clark were obtained from the crops research division, Agricultural Research Center, Giza, Egypt.

Irradiation source and technique:

The source of radiation was ^{60}Co gamma cell 4000, at the National Center for Research and Radiation Technology, Nasr City, Cairo, Egypt.

Groups of seeds (50 of each) were placed into small plastic bags. These bags were placed in the irradiation chamber of the gamma cell. The dose rate range was 0.23 Gy/second. The dose levels used for soybean seeds were 5, 10, 20, 30, 40, 50, 80 and 100 Gy.

Methods

Experimental area:

The experimental studies were carried out at Atomic Energy Authority, Hot Laboratories Center, Inshas, Egypt. The experimental area was ploughed twice ridged and divided into plots. Each plot consisted of 6 rows, 6m long and 50 cm apart to represent the system of corn or soybean sowing with 3 rows. The plot area was left between plots to avoid overlapping or shading effects. The soil was irrigated immediately after sowing the seeds. The first irrigation was carried out 10 days after sowing, while the following regularly took place 5 days intervals.

Soil type

The soil used in all experiments was subjected to the mechanical analysis method of Piper (1950) at the soil department, Faculty of Agriculture, Cairo University, Giza. Data was performed in Table (1).

Table (1): Mechanical analysis of soil

% particle size distribution				
Coarse sand	Fine sand	Silt	Clay	Texture class
2.70	33.00	28.80	35.35	Clay loam

Germination test:

This test was done at laboratory scale in order to examine the effect of gamma irradiation on germination of soybean seeds. Seed samples were exposed separately to ^{60}Co gamma radiation with doses of 5, 10, 20, 30,

40, 50, 80 and 100 Gy for soybean seeds. Ten seeds from each treatment were germinated in a 9 cm Petri dishes moistened with 5 ml of distilled water. Moistening of the filter paper was repeated after 2 days with 2 ml of distilled water. The Petri dishes were incubated at 30˚C in the darkness for germination. Seeds with visible radicals were counted. The parameters recorded were final germination percentage and days required reaching the final germination percentage.

Chemical analysis:

After 46 days from germination, soybean leaves were chemically analyzed. The analysis includes determination of chlorophyll a, chlorophyll b and carotenoids. Total carbohydrates, nucleic acids, total protein were also determined.

Chlorophyll a, b and carotenoids were determined according to the method of Metzner *et al* (1965). Total hydrolysable carbohydrates and total soluble sugars were estimated according to the method of Dubois *et al* (1956). Reducing sugars were determined using the dinitro salicylic acid method according to Miller (1959). Non reducing sugars were calculated by difference between the total soluble sugars and the reducing sugars. Nucleic acids were determined by the method of Astawrov (1974). Crude protein was determined according to the method of A.O.A.C. (2000)

RESULTS AND DISCUSSION

Effect of gamma irradiation with various doses on germination percentage of soybean seeds:

The results given in Table (2) and illustrated in Fig. (1) showed the effect of gamma irradiation with various doses on germination percentage of soybean seeds. The results cleared that, the maximum germination percentage at 40 Gy treatment was 100% after 4 days of germination compared with control (81%), while 20 and 30 Gy treatments gave maximum percentage of germination of 100% after 5 days of germination compared to control (93%). These findings were in a good agreement with Olfat *et al* (1985) and Seilm and El-Banna (2001) who found that, irradiation of soybean with high doses of gamma source caused failure in germination, while lower doses stimulated it.

Table (2): Effect of gamma irradiation with various doses on germination percentage of soybean seeds.

Dose (Gy)	Germination percentage in days				
	1 day	2 days	3 days	4 days	5 days
Control	0	33 ± 1.0^{D}	72 ± 0.95^{C}	81 ± 1.0^{E}	93 ± 1.0^{C}
5	0	40 ± 1.0^{C}	81 ± 0.95^{B}	95 ± 1.0^{CD}	96 ± 1.0^{BC}
10	0	50 ± 1.0^{B}	83 ± 0.95^{B}	96 ± 1.0^{BC}	99 ± 1.0^{AB}
20	0	50 ± 1.0^{B}	89 ± 0.95^{A}	99 ± 1.0^{AB}	100 ± 1.0^{A}
30	0	60 ± 1.0^{A}	80 ± 0.95^{B}	92 ± 1.0^{D}	100 ± 1.0^{A}
40	0	60 ± 1.0^{A}	90 ± 0.95^{A}	100 ± 1.0^{A}	100 ± 1.0^{A}
50	0	42 ± 1.0^{C}	61 ± 0.95^{D}	92 ± 1.0^{D}	93 ± 1.0^{C}
80	0	30 ± 1.0^{D}	30 ± 0.95^{E}	40 ± 1.0^{F}	40 ± 1.0^{D}
100	0	20 ± 1.0^{E}	20 ± 0.95^{F}	20 ± 1.0^{G}	30 ± 1.0^{E}

Values with different superscript within parameter are significantly differ, p<0.05 NS means values ±SE. Each value is a mean of two seasons.

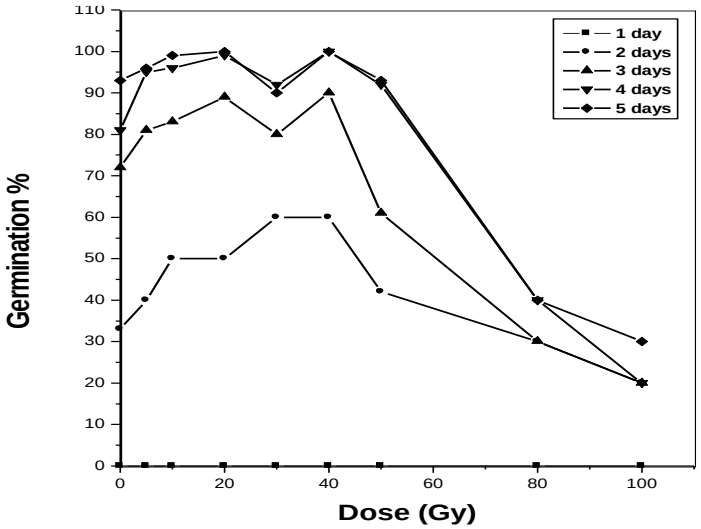

Fig. (1) Effect of gamma irradiation with various doses on germination percentage of soybean seeds

Effect of gamma irradiation with various doses on the photosynthetic pigments of soybean leaves:

The effect of gamma irradiation on the photosynthetic pigments of soybean leaves are given in Table (3) and illustrated by Fig. (2).

Table (3): Effect of gamma irradiation with various doses on photo-synthetic pigments of soybean leaves (mg/g) after 46 days from germination.

Dose (Gy)	Chlorophyll a	Chlorophyll b	Carotenoids
Control	26.06±0.03^{G}	1.85±0.03^{E}	16.72±0.03^{F}
5	31.10 ±0.04^{D}	2.38±0.03^{C}	18.92±0.03^{D}
10	30.90±0.04^{E}	2.28±0.03^{C}	18.96±0.03^{D}
20	37.84±0.04^{C}	3.38±0.03^{B}	23.96±0.03^{C}
30	40.84±0.04^{B}	3.30±0.03^{B}	31.46±0.03^{B}
40	44.26±0.04^{A}	5.12±0.03^{A}	33.12±0.03^{A}
50	28.58±0.04^{F}	2.14±0.03^{D}	18.40±0.03^{E}
80	21.66±0.04^{H}	1.41±0.03^{F}	15.54±0.03^{G}
100	18.66±0.04^{I}	1.34±0.03^{F}	13.38±0.03^{H}

Values with different superscript within parameter are significantly differ, p<0.05 NS means values ±SE. Each value is a mean of two seasons.

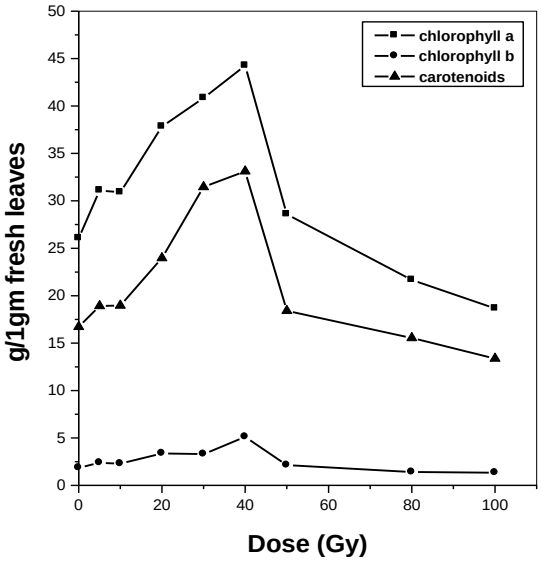

Fig. (2) Effect of gamma irradiation with various doses on photosynthetic pigments of soybean leaves after 46 days from germination

The obtained results cleared that, the highest chlorophyll a, b and carotenoid contents were detected with dose level of 40 Gy. The amounts were 44.26, 5.12 and 33.12 mg/g leaves respectively as compared to control. These findings were in accordance with those reported by Tai-Sheng and Joel (1999) who revealed that, the total chlorophyll content was reduced by 70-80% at doses of gamma rays above 100Gy. The reduction in total chlorophyll content may relate to loss of certain photosynthetic complexes.

Effect of gamma irradiation with various doses on carbohydrate contents of soybean leaves:

Data of the effect of gamma irradiation on the carbohydrate contents of soybean leaves are given in Table (4) and illustrated by Fig.(3). These results were in a good agreement with those reported by Yonies (1997) who stated that soybean seeds which irradiated with gamma rays had a significant change in the carbohydrate contents in the investigated seeds. Also, the present results agreed to a large extent with those reported by Inayatullah *et al* (1988) who found that gamma irradiation at doses of 0.25, 0.50, 1.00, 2.00 and 500 KGy had a significant effect on chemical components such as carbohydrates of 5 varieties of soybean seeds.

Table (4): Effect of gamma irradiation with various doses on carbohydrate contents percentage of soybean leaves after 46 days from germination.

Dose (Gy)	Total hydrolysable carbohydrates	Total soluble sugars	Reducing sugars	Non-Reducing sugars
Control	23.18±0.01^{E}	2.39±0.01^{F}	0.12±0.01^{B}	2.27±0.03^{D}
5	23.21±0.01^{E}	2.51±0.01^{E}	0.19±0.01^{C}	2.32±0.03^{D}
10	23.97±0.01^{D}	2.79±0.01^{D}	1.23±0.01^{B}	2.56±0.03^{C}
20	24.36±0.01^{C}	2.96±0.01^{C}	0.26±0.01^{B}	2.70±0.03^{B}
30	24.89±0.01^{B}	3.15±0.01^{B}	0.31±0.01^{A}	2.84±0.03^{A}
40	25.52±0.01^{A}	3.21±0.01^{A}	0.33±0.01^{A}	2.88±0.03^{A}
50	24.33±0.01^{C}	2.81±0.01^{D}	0.23±0.01^{B}	2.58±0.03^{C}
80	22.23±0.01^{F}	1.15±0.01^{G}	0.09±0.01^{D}	1.06±0.03^{E}
100	20.86±0.01^{G}	0.98±0.01^{H}	0.03±0.01^{E}	0.95±0.03^{E}

Values with different superscript within parameter are significantly differ, p<0.05 NS means values ±SE. Each value is a % of dry weight and a mean of two seasons.

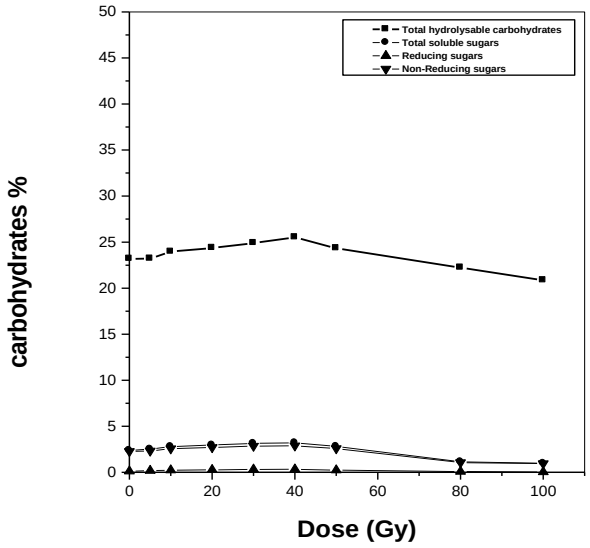

Fig. (3) Effect of gamma irradiation with various doses on carbohydrates contents percentage of soybean leaves after 46 days from germination

Effect of gamma irradiation with various doses on nucleic acid contents of soybean leaves:

The effect of gamma irradiation with different doses on DNA and RNA contents of soybean leaves after 46 days from germination are given in Table (5) and illustrated in Fig.(4).

Table (5): Effect of gamma irradiation with various doses on the amount of DNA and RNA (µg/g fresh weight) in soybean leaves after 46 days from germination.

Dose (Gy)	DNA	RNA
Control	18.20±0.09^{C}	44.30±0.05^{F}
5	18.30±0.09^{C}	44.41±0.05^{F}
10	18.90±0.09^{B}	49.60±0.05^{E}
20	19.20±0.09^{AB}	55.11±0.05^{C}
30	19.28±0.09^{A}	58.70±0.05^{B}
40	19.40±0.09^{A}	61.13±0.05^{A}
50	18.10±0.09^{C}	51.08±0.05^{D}
80	16.80±0.09^{D}	43.22±0.05^{G}
100	14.70±0.09^{E}	31.23±0.05^{H}

Values with different superscript within parameter are significantly differ, p<0.05 NS means values ±SE. Each value is a mean of two seasons.

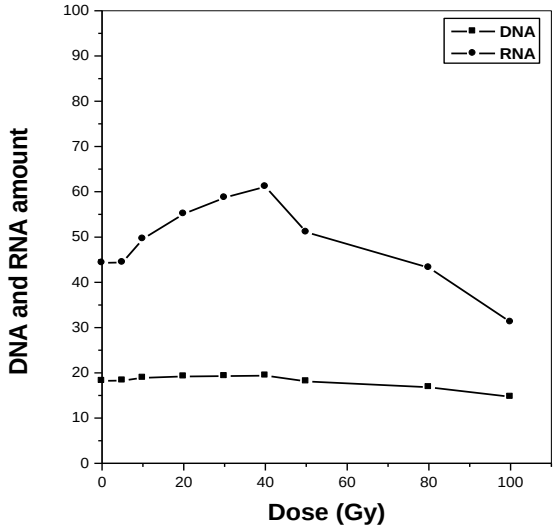

Fig. (4) Effect of gamma irradiation with various doses on nucleic acids contents of soybean leaves after 46 days from germination

The obtained results indicated that, the highest level of DNA and RNA contents were obtained at dose level of 40 Gy. However, lowest ones were observed at dose 100 Gy as compared to control group. The amounts of DNA and RNA for treated seeds with 40 Gy were 19.40 and 61.13 µg/g of fresh soybean leaves respectively, while the amounts of DNA and RNA were 14.70 and 31.23 µg/g respectively for dose of 100 Gy. The DNA and RNA contents for control group were 18.20 and 44.30 µg/g of fresh leaves respectively. These results conformed with those reported by Kanazawa *et al* (1998) who indicated that, there was a significant decrease in the amount

of DNA after gamma irradiation of tobacco plants at dose level of 0-300 Gy, this was due to cellular system that were induced by irradiation and resulted in enzymatic degradation of DNA.

Effect of gamma irradiation with various doses on protein percentage of soybean leaves:

The effect of gamma irradiation with various doses on protein percentage of soybean leaves after 46 days from germination are given in Table (6) and illustrated by Fig. (5).

Table (6): Effect of gamma irradiation with various doses on protein percentage of soybean leaves after 46 days from germination.

Dose (Gy)	Protein %
Control	22.38±0.01^{F}
5	22.72±0.01^{E}
10	23.11±0.01^{C}
20	23.04±0.01^{D}
30	23.63±0.01^{B}
40	23.74±0.01^{A}
50	22.07±0.01^{G}
80	20.15±0.01^{H}
100	17.05±0.01^{I}

Values with different superscript within parameter are significantly differ, p<0.05 NS means values ±SE. Each value is a % of dry weight and a mean of two seasons.

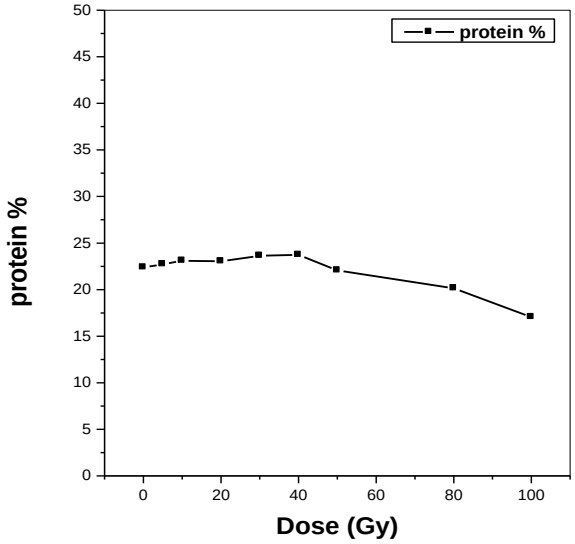

Fig. (5) Effect of gamma irradiation with various doses on protein percentage of soybean leaves after 46 days from germination

The results cleared that, the maximum protein content was observed at the dose level 40 Gy in comparison with the control treatment followed by 30, 20, 10 and 5 Gy in descending order. These results were in good agreement with those reported by Ragab (1988) who cleared that, exposing lupine seeds to various doses of gamma radiation influenced the synthesis of protein in plant leaves after irradiation.

CONCLUSION

It may be concluded that, the use of gamma irradiation with dose 40 Gy significantly increased germination percentage, photosynthetic pigments, carbohydrate contents, nucleic acids and protein percentages of soybean leaves. However, at higher doses the chemical constituents of the leaves and germination percentage were markedly decreased.

It could be recommended to use 40 Gy for soybean crop because this dose gave the highest yield of seeds by activating germination and biosynthesis processes during the growth of the plants.

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تأثير التشعيع الجامى على الإنبات والمحتوى الكيميائى الحيوى لأوراق نبات فول الصويا

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يتناول هذا البحث دراسة تأثير التشعيع الجامى للكوبالت 60 بجرعات تتراوح ما بين 5 – 100 جراى لبذور فول الصويا وذلك على نسبة الإنبات ، الكلورفيل أ، ب، المحتوى الكربوهيدراتى، الاحماض النووية والبروتينات لأوراق نبات فول الصويا.

وقد أوضحت النتائج أن أعلى نسبة للإنبات وصلت إلى 100% فى بذور فول الصويا عند الجرعة 40 جراى مع زيادة الكلورفيل أ، ب والكاروتينات عند نفس الجرعة إلى جانب تنشيط للمحتوى الكربوهيدراتى والاحماض النووية والبروتينات فى النبات.