# **Stimulatory Effects of Low Ionizing Radiation on Plant**

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### 1. Introduction

Generally, "hormesis" comes from "hormo" which mean 'to excite' in Greek. It's understood that the hormesis effect is subharmful doses of any harmful agent may evoke a stimulatory response in any organism irrespective of kind of agent. Also, Arndt-Schultz Law in the toxicology as small doses of poisons are stimulatory has the same meaning. In the radiation hormesis it is considered that radiation is also one of these agents<sup>1</sup>.

Based on the investigation of irradiation experiments with various animals and plants carried out between 1930's and 1970's, in 1982 T. D. Luckey of Department of the biochemistry, Missouri-Columbia University led to the conclusion that low level radiation evokes a stimulatory effect in the body. He explicated the concept for radiation hormesis of low level radiation<sup>2</sup>). At that time, the radiation biology field was ruled by the paradigm<sup>3</sup> that 1) irradiation exposure is harmful, 2) how small doses is irradiation exposure is harmful, 3) there is not influence of low dose radiation that cannot estimate by studies for influence of high dose radiation. However, the concept of radiation hormesis effect by Luckey is as follows: 1) biopositive effects of low dose radiation; influence caused by low dose radiation is totally different from one caused by high dose radiation, low dose radiation produces physiological useful effect against high dose radiation. 2) Radio-adaptive response; radiation also acts the organism as stress. Previously small dose radiation raises defense response against the stress (radiation), resulting in the phenomenon that radiation influence decreases in appearance. As a biopositive effect, in the former concept Luckey shows a schematic diagram of the relation between absorbed dose rate and the hormesis response as shown in Figure 1. In the Figure ure, the base response of 100 percent is equivalent to response to background radiation. The response at over the base percentage (100%) shows a bio-positive effect, on the contrary the response below shows a harmful influence, namely, bio-negative effect (including the death).





The dose rate value at which the response quantity in the organism becomes equivalent to a case irradiated with only background radiation is called Zero Equivalent Point; ZEP. On the dose rate, a balance is kept between the bio-positive and bio-negative effects. This means that the effect on the ZEP in the organism is equivalent to the effect in background radiation environment. At the left area from the ZEP, the bio-positive effect is seen, namely, the radiation hormesis effect can be expected.

After Luckey proposed the concept of radiation hormesis, studies on the effects have been carried out with a lot of animals such as virus, bacteria, protozoan, invertebrate animal, vertebrate animal and plants. Especially for plants, many studies have been carries out, since the dose range possible to observe this stimulatory response is from a few cGy to several tens Gy and in wider than the range for animals (from a few cGy to several tens cGy)<sup>4</sup>, and a number of experimental samples can be obtained easily. However, in the previous studies there are many problems as follows: the dose estimation of radiation is not clear, experimental reproducibility is very poor. To precisely investigate the mechanism of the hormesis phenomenon, it is necessary to obtain a lot of systematical experimental data and to analyze the data very carefully. Also, though almost all the results for plants were made using of gamma-ray and X-ray, we think that investigation with other kinds of radiation such as neutron is quite important, it is thought that neutron has a very large reactivity on the radiation hormesis by comparing with other radiations.

This paper presents the investigating result for the phenomenon of radiation hormesis effect for plants irradiated with fusion neutrons, fission neutrons and <sup>60</sup>Co gamma-rays, to explicate the mechanism of radiation hormesis effect.

#### 2. Experiment and analysis

The present research approach consists of 4 processes, irradiation, cultivation, measurement and statistical analysis. The schematic procedure is in Figure 2.



# 2-1 Irradiation sample

As an irradiation sample we selected dry seeds of Raphanus sativus for the following reasons. In plants the seed is the most stable in the growth stage. Seeds are small and easily transported. The reason why we used dry seeds of Raphanus sativus are used is as follows. The period of growth is short. The difference individuals after their growth are small in comparison with other plants. And seeds germinate a few days; the height of seed leaf hypocotyls becomes 10 cm in a week. In addition it can be cultivated by hydroponics, so we can keep conditions constant easily.

# 2-2 Radiation Sources and Irradiation methods

In this experiment dry seeds of Raphanus sativus were irradiated with D-T neutrons, thermal and fast neutrons in nuclear reactor and <sup>60</sup>Co gamma-ray, because the experiment with neutrons and gamma-rays can find out the differences of radiation effects depending on radiation quality. The other reason is that in a neutron field secondary gamma-rays are easily produced by neutron interactions with surrounding materials, so the effects of secondary gamma-rays can be estimated from the irradiation experiment with <sup>60</sup>Co.

#### 2-2-1 Neutron Irradiation

Irradiation of fusion (D-T) neutrons was done at the Intense 14 MeV Neutron Source Facility OKTAVIAN of Osaka University and Fusion Neutron Source (FNS) of Japan Atomic Energy Research Institute. And irradiation of thermal and fast neutrons was done in Atomic Energy Research Institute Reactor UTR-KINKI of Kinki University.

# 2-2-2 Gamma-ray Irradiation

Irradiation of <sup>60</sup>Co gamma-rays was done with <sup>60</sup>Co gamma-ray source of Radio Isotope Research Center and the Institute Scientific and Industrial Research of Osaka University, the radiation doses of which are ranging from  $5\mu$ Gy to10mGy and  $5\mu$ Gy to10mGy, respectively.

# 2-3 Estimation of Absorbed Dose

The neutron flux was determined with foil activation method. Foil activation method is suitable to

measurement of neutron flux for this experiment, because it enables to measure the neutron fluence in a minute space and dose not disturb the neutron field. However, the irradiation field has an energy spectrum. To estimate an exposure dose, we measured the neutron energy spectrum by activation foil detector. In this experiment activated foils were measured by a high purity germanium detector.

In a nuclear reactor, there is a weak angular dependence in neutron irradiation at the sample position and operation with various thermal powers causes a significant change in dose rate. The thermoluminescence dosimeter (TLD) was used for the measurement of gamma-ray dose because its volume, direction dependence and dose rate dependence are small.

Figure 4 shows the relative effectiveness for height of a hypocotyls and total weight of a seed leaf as a function of exposure dose for these radiation fields. For the fusion neutron irradiation experiment, the conversion factor from neutron fluence to absorbed dose was calculated with the Monte Carlo code MCNP-4a and JENDL-3.2 as a nuclear data library. In the calculation, it was assumed that the neutron source spectrum of OKTAVIAN could be used and Raphanus sativus seed contained equal amount of carbon, hydrogen, oxygen and nitrogen.

# 2-4 Cultivation and Measurement Method

Cultivation was done with hydroponics. Generally gauze is used in growing the Raphanus sativus. But roots get entangled to the gauze and it is unable to separate them. In this experiment the length of root is one of the measurement subjects, so filter papers, which hardly make roots entangled, were used instead of gauze.

In parallel with irradiated seeds non-irradiated seeds were cultivated in the same room. To keep the environment condition constant, light was shielded and room temperature was controlled with an air conditioner. The growth environment was kept uniformly as much as possible.

Seed leaves were measured at 7th days after starting cultivation. Measurement subjects are length of a hypocotyls, length of a root full length of a seed leaf, total weight of a seed leaf and germination rate. We defined the term "length of a hypocotyls" as the length from the joint of a root to the joint of a leaf and the term "length of a root" as the length from the joint of a root.

# 2-5 Statistical Analysis of Measurement Data

We defined an index of the radiation hormesis effect as a ratio between the mean measured values of irradiated and non-irradiated samples. Namely the relative effect is express as the following equation.

#### ( mean value of irradiated sample )

Relative Effectiveness = (mean value of non-irradiated sample)

On the equation relative effect of unity means there is no irradiated effect in appearance; the effect of irradiated seed is equal to that of non-irradiated seed, the relative effectiveness of the weight, 1.2, means that the average weight of the irradiated group became 1.2 times heavier than that of the non-irradiated group.

# 3 Results

# 3-1 60Co Gamma-ray

Figure 3(a) shows the relative effectiveness for height of a hypocotyls and total weight of a seed leaf as a function of exposure dose. At the range from 10-5Gy to 10-1Gy the relative effectiveness for both was almost unity. But same as germination rate, each relative effectiveness was from 0.6 to 0.8 at the range between  $10^{3}$ Gy and  $10^{4}$ Gy, and at the range over  $10^{4}$ Gy there was no seed germination.

# 3-2 Fission Neutron

Figure 3(b) shows the relative effectiveness for height of a hypocotyls and total weight of a seed leaf as a function of exposure dose. In this experiment we measured neutron fluence only for thermal and fast (around a few MeV energy) neutrons. At the range from  $1 \times 10^7$ Gy to  $2 \times 10^{11}$ Gy, the relative effectiveness for length of a hypocotyls and total weight was almost unity.

# 3-3 Fusion Neutron

Figure 3(c) shows the relative effectiveness for height of a hypocotyl and total weight of a seed leaf as a function of exposure dose. In fusion (D-T) neutron irradiation experiment, unlike other two radiation fields, the relative effectiveness for total weight of a seed leaf was over 1.0~1.2 at maximum at the range from  $1 \times 10^8$  n/cm<sup>2</sup> to  $1 \times 10^{10}$  n/cm<sup>2</sup>. Also, relative effectiveness was 0.5 in  $10^{13}$  n/cm<sup>2</sup> and there was no seed germination in the range over  $10^{14}$  n/cm<sup>2</sup>.

Using the conversion factor in Sec.2-3 we got this Figure about the relation of relative effectiveness to exposure dose. As a result, we can find radiation increase effect at range from 1cGy to 10Gy.



Figure 3(a). The distribution of the relative effectiveness for the length of hypocotyl and the total weight of a seed leaf against the absorbed dose by <sup>60</sup>Co gamma-ray

Figure 3(b). The distribution of the relative effectiveness for the length of hypocotyl and the total weight of a seed leaf against the neutron fluence of thermal and fast neutron

# 0 1.00E+05 1.00E+07 1.00E+09 1.00E+11 1.00E+13 1.00E+15 Neutron fluence (n/cm^2)

Figure 3(c). The distribution of the relative effectiveness for the length of hypocotyl and the total weight of a seed leaf against the neutron fluence of **D-T** neutron

#### 4 Discussion

#### 4-1 Welch's Test

Figure 5 shows the frequency distribution of the total weight of seed leaf for two irradiated seeds having increase and decrease effects and non-irradiated seeds. It's found from Figure 5 that all the histograms have the same shape. This means that the distribution of non-irradiated seeds just shift by irradiation to yield those of irradiated seeds and as a result the mean weight increase (decrease). The welch's test was carried out to confirm that there is a significant difference of mean weight between irradiated samples and non-irradiated samples. We can find the difference from level of significance  $\alpha = 0.05$ .



Figure 4. The comparison of histogram for the total weight of a seed leaf between non-irradiated and irradiated seeds with D-T neutron on the occasion of indicating of hormesis effect

# 4-2-1 Approach to the mechanism

In this experiment, positive effectiveness was found only for D-T neutron, not thermal and fast neutrons and <sup>60</sup>Co gamma-rays. These results probably do not depend on only absorbed energy of radiation. We expect that there is some interaction during the process that radiation gives energy to material atoms. We think that such a interaction should be based on some threshold nuclear reactions because threshold nuclear reactions can be induced only by fast neutrons such as D-T neutrons. Practically, nuclear reactions of neutrons with hydrogen (H), carbon (C), oxygen (O), nitrogen (N) and phosphorus (P) (principal ingredient in the seed) have to be taken into account. It can be confirmed from the irradiation D-D neutron experiment whether the threshold nuclear reaction causes the effectiveness. Table.1 shows threshold neutron reaction neutron cross sections of H, C, O, N and P.

The table shows that for the D-T neutron the cross section of the (n,a) reaction is larger than that of the (n,p) reaction for all the nuclei. It is thus expected that the (n,a) reaction contributes greatly to the mechanism of the increase effect. For the D-T neutron, because of the threshold energy, both the (n,p) and (n,a) reactions do not occur for <sup>12</sup>C and <sup>16</sup>O. In respect to N, though both (n,p) and (n,a) reactions by D-D neutrons can be included, since its composition ratio is relatively small, it seems that they can be negligible comparing that of (n,a) reactions by D-T neutrons. Therefore, if the increase effect cannot be found in the D-D neutron irradiation, it is though that (n,a) reactions may cause the effect. If the effect is found, the energy transfer by means of recoiled protons can be play an important role. Because the cross section of elastic scattering of H is large and it seems that numerous H atoms exist in a seed, it can be expected that the interaction of H nuclei and incident neutrons concerns the effect and can find out the mechanism of the increase effect.

nuclei	kind of reaction	Threshold value	D-D neutron 2.45 MeV	D-T neutron 14 MeV
$^{1}\mathrm{H}$	elastic scattering	3 <del>1-3</del> 5		692 mb
<sup>12</sup> C	(n,p)	13.8 MeV		164 µ b
	(n,α)	6.3 MeV		72.65 mb
<sup>16</sup> O	(n,p)	11.2 MeV		43.73 mb
	(n,α)	3.8 MeV		109 mb
$^{14}N$	(n,p)		10-100 mb	4.2 mb
	(n,α)	1.3 MeV	100 mb	60.1 mb
<sup>31</sup> P	(n,p)	1.2 MeV	50 mb	142 mb
	(n,α)	4.1 MeV		121 mb
	(n,n p)	8.2 MeV	<del></del>	374 mb
	(n, n α)	12.2 MeV		13.8 mb
	(n,2n)	13.3 MeV		16 mb

Table 1. threshold neutron reaction neutron cross sections of H, C, O, N and P

# 4-2-2 Irradiation experiment with D-D neutrons

The D-D neutron (2.45MeV) irradiation experiment was carried out to confirm the hypothesis in the previous section. The irradiating experiment was done at OKTAVIAN. The range of irradiation dose was from X  $n/cm^2$  to X  $n/cm^2$ . The preliminary results are described in the following.

Figure 6 shows the relation of the absorbed dose to relative effectiveness for the length of hypocotyls, the length of the roots, the full length and the total weight. In the experimental result of D-D neutron irradiation, the increase effects of 23% in length of root and 13% in full length were found. On the other hand, as far the total weight the effectiveness was approximately unity, although the prominent increase could be found in the case of D-T neutrons. Figure 7 shows the relation of the absorbed dose of all the radiations to relative effectiveness for the total weight. In the present results the range of D-D neutron fluence is narrow compared with other radiations. Therefore, the measured data in D-D neutrons experiment are insufficient to discuss and prove the hypothesis introduced in Sec.4-2-1 and it is necessary to obtain more significant data.



Figure 6(a). The distribution of the relative effectiveness for the length of hypocotyl and the total weight of a seed leaf against the neutron fluence of D-D neutron



Figure 6(b). The distribution of the relative effectiveness for the length of root and the full length of a seed leaf against the neutron fluence of D-D neutron

Figure 7. The distribution of the relative effectiveness for the total weight of a seed leaf against the absorbed dose by the radiation

#### 5. Conclusion

Irradiation experiment of dry seed of Raphanus sativus has been carried out at broad dose range with fusion (D-T) neutrons, thermal and fast neutrons and <sup>60</sup>Co gamma-rays. Possible effectiveness has measured for the length of a root, the length of hypocotyls, full length of a seed leaf, total weight of a seed leaf and germination rate. The positive effectiveness was observed only in the case of D-T fusion neutron irradiation. This effectiveness was from 5% to 25% and could be observed at the range from 1cGy to several tens Gy. We think this phenomenon may be due to the hormesis effect.

In irradiation with the D-D neutron, positive effectiveness for the length of a hypocotyls and root was observed though the result is preliminary. But the mechanism was not still made clear because the experimental data are insufficient

Further investigation is necessary especially for D-D neutron irradiation experiment to find out the mechanism of the radiation hormesis.

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