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IDENTIFICATION METHODS FOR IRRADIATED WHEAT

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Identification Methods for Irradiated Wheat

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The effect of irradiation on wheat seeds was examined using various kinds of analytical methods for the identification of irradiated seeds.

In germination test, the growth of sprouts was markedly inhibited at 500Gy, which was not affected by storage. The decrease in germination percentage was detected at 3300Gy. The results of enzymatic activity change in the germ measured by Vita-Scope germinator showed that the seeds irradiated at 10kGy could be identified. The content of amino acids in ungerminated and germinated seeds were analyzed. Irradiation at 10kGy caused the decrease of lysine content but the change was small which need very careful operation to detect it. The chemiluminescence intensity increased with radiation dose and decreased during storage. The wheat irradiated at 10kGy could be identified even after 3 months storage. In the electron spin resonance (ESR) spectrum analysis, the signal intensity with the g value of 2.0055 of skinned wheat seeds increased with radiation dose.

Among these methods, germination test was the most sensitive and effective for identification of irradiated wheat.

Keywords: Wheat, Identification, Germination, Chemiluminescence, Amino Acids, ESR, Irradiation

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照射小麦の検知法

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（1992年1月24日受理）

照射小麦の検知法の開発を目的として，小麦の照射による種々の変化について検討した。

発芽試験では，芽の伸長が500Gy で著しく阻害され，貯蔵中も変化しなかった。発芽率の減少は，3300Gyで検出された。胚芽の酵素活性を発芽試験装置（バイタスコープ）で測定した場合には，10kGy照射で検知できた。アミノ酸分析では，10kGyでリジンの減少が認められたが，わずかな変化であり検知に用いるのは難しいと考えられた。化学発光強度は，照射線量に比例して高くなったが，貯蔵中に減少した。10kGy照射した場合には，3ケ月貯蔵後でも検知できた。ＥＳＲ測定では，皮を険いた小麦のｇ値 2.0055 におけるシグナルが線量に比例して増加した。

これらの方法の中で，発芽試験法が最も感度がよく，照射小麦の検知法として効果的であった。

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1. INTRODUCTION

The treatment of food by ionizing radiation has been studied to improve the storage quality and decreasing the risk of food poisoning from pathogenic microorganisms. In 1980 the Joint FAO/IAEA/WHO Expert Committee concluded that irradiation of any food commodity up to an average dose of 10 kGy causes no toxicological hazard and introduces no special nutritional or microbiological problems (1). More than 30 governments have regulations allowing the use of irradiation for processing special food commodities (2), but in other countries the irradiation of food is prohibited. The commercial application of this technology is limited since there are some psychological problems of consumers for safety concerning and technological problems for detecting irradiated food.

International Conference on the Acceptance, Control of, and Trade in Irradiated Food in Geneva, December 1988, reached the conclusion that "It is well known that the changes associated with food irradiation are difficult to detect. However, it is recognized that detection methods, if available, would augment standard regulatory procedures and would thereby help to assure consumers that processors and distributors are adhering to government control procedures. Research on detection methodology should be continued." The conference also recognized that "Consumer confidence can be bolstered when there is clear evidence that the food irradiation process is being effectively controlled by responsible industry and a governmental regulatory

process".

It is well known that the changes associated with food irradiation are difficult to detect because the changes in the statutory dose range are small. Sometimes the changes are very similar to the changes which occur upon other food preservation treatment. Recent years some methods for identification of irradiated food, based on the changes occurred in irradiated food, have been proposed (3). There are physical, chemical, histological and morphological, and microbiological methods. Usually each method is suitable for identification of special food on which the method developed. The dose of radiation applied is dependent on its purpose. For the commercial application, the lowest dose levels (0.1 to 1 kGy) are used for sprouting inhibition, intermediate levels (1 to 10 kGy) for disinfestation or delaying ripening, and the highest levels (10 kGy to 50 kGy) for the disinfection of microorganisms. For wheat the dose commonly used is 1 to 10 kGy.

In this research, the effect of irradiation on wheat seeds was examined using various kinds of analytical methods for the identification of irradiated seeds.

2. MATERIALS AND METHODS

2.1 Samples

Wheat seeds (Nohrin No.61) used in this experiment were donated by Gunma Agricultural Research Center. The seeds harvested in 1988 were mainly used in this paper. When the seeds harvested in 1984 were used for comparison, the seeds in 1988 and 1984 were called new seed and old seed, respectively.

2.2 Irradiation

Seeds were irradiated in polyethylene bag at room temperature using cobalt-60 slab source. The dose rate used was 1 to 10 kGy/h determined by Fricke dosimetry.

2.3 Germination and growth of seeds

Twenty-five to fifty grains of seed were placed in 9 cm Petri dishes on a 8-layers paper pad moistened with distilled water, and incubated at room temperature (ca. 28°C) for 5 days. Germination percentage and the length of sprouts and roots were measured on the last day. Germination was defined as the protrusion of both the sprout and root to the extent of at least 3 mm in length. The data of length was arranged from longest to shortest. The first 60% of data were used to calculate the mean and standard deviation (SD).

2.4 Enzymatic activity for germination

The enzymatic activity for germination of wheat seeds was

measured by Vita-Scope germinator Foss Electric, Denmark). Seeds were cut half and half at the germ, then put them into the Vita-Scope. The hydrogen produced by the action of enzyme will react with the tetrazolium solution in Vita-Scope and produce a red color. Theoretically, if no enzymatic activity exists, no color is produced. In fact the staining of germ in red color had different types. If the red color covered more than half the germ, the seeds was judged alive. If the color was light or the color covered less than half the germ, the seeds was judged dead.

2.5 Analysis of amino acids

The content of amino acids was analyzed in germinated and ungerminated seeds after irradiation. Ungerminated seeds were ground into powder, and 50 mg of powder was mixed with 1 ml 6 N HCl for hydrolysis. One gram seeds was germinated in distilled water for 4 days and then homogenized with water at the total weight of 30 g. The homogenate was mixed with the same volume of concentrate HCl for hydrolysis.

Hydrolysis was performed in air-evacuated and sealed tubes at 110°C for 22 h by AL-500D Dry Block Bath (SCINICS Co. LTD., Tokyo). Hydrolytic liquid was filtered with filter paper, followed by a rinse, and then dried in vacuum. One ml lithium citrate buffer was used to dissolve the residue, and then filtrated with microporous filter of 0.45 μm. An injection of 10 μl sample solution was analyzed by IRICA model Sigma-8700 Amino Acid analyzer with a column of anion exchange resin IRICA SCX-1005 (Irika Instruments Inc., Kyoto).

2.6 Chemiluminescence

The measurement of chemiluminescence (CL) of 2.70 g seeds (one layer on the bottom of light-measuring cell) was performed on Chemiluminescence Analyzer OX-7 (TOHOKU Eelectronic Industrial Co. LTD., Sendai) at room temperature with gate time 60 seconds. First the intensity was measured in dry condition, and then some liquid was added to let the seeds germinate. Liquids and light-measuring cell were cooled in/on ice in advance.

2.7 Electron Spin Resonance

Irradiated seeds were skinned by the peeler (Kett Science Co. Ltd., Tokyo) for 4 min and eight grains were used for ESR analysis. ESR measurement was performed by Japan Electric Company using JEOL-RE2X and ES plit 330 computer system.

3. RESULTS AND DISCUSSION

3.1 Germination and growth

Seed growth is a consecutive process. In the first two days most of seeds split their skin and protruded the bud. Some seeds had a bud about 0.5 to 1.5 mm, then the growth stopped. In fact it is difficult to say how long the bud should be in germinated seeds when the protrusion increased little by little. In this paper, germination was defined arbitrarily as above.

The result of germination percentage, sprout length and root length of new seeds were shown in Table 1. Germination percentage was significantly decreased at 3.3 kGy. At 10 kGy, germination was almost completely inhibited. Many seeds in 6.6 kGy and 10 kGy groups shed out endosperms from germ. Sprout length and root length were measured to see the effect of irradiation on growth rate. Because some seeds did not germinate and some did not grow, it is better to use full growth seeds to evaluate growth rate. In this experiment the first 60% of seeds which had longest sprout and root were used to calculate the mean value and standard deviation (SD) of sprout length and root length. The statistical conclusion on sprout length was consistent with root length. Significant difference was found at 0.33 kGy ($p<0.01$).

Table 2 shows the results of the low dose irradiation of wheat seeds at room temperature. The results of low dose irradiation at 0°C were also the same as at room temperature (data not shown). Low dose irradiation less than 500 Gy did not

affect the germination percentage. Storage of the seeds for some years not only reduced germination percentage, but also delayed germination. The germination time of old seeds was about 1 day delayed to that of new seeds. The sprout length of old seeds was the same as new seeds when the germination in old seeds was 1 day elongated. At the dose of 500 Gy or more, growth was seriously inhibited ($p<0.01$), which could not be improved by elongation of germination. There was a tendency that low dose irradiation promotes seeds growth but the effect was small. Figure 1 shows the distribution of sprout length of new seeds after 5 days germination. The length of seeds irradiated at 1 Gy and 10 Gy was a little longer than that of control. Most of the seeds had sprouts longer than 60 mm. The length at 100 Gy was similar to control. At 330 Gy, the distribution of length was changed and a few seeds had the sprouts longer than 60 mm. No seed had a sprout longer than 30 mm in 500 Gy group.

The sprout length of old seeds was significantly shorter than that of new seeds (Table 2). Therefore, it was difficult to distinguish the unirradiated old seeds from the new seeds irradiated at 330 Gy by sprout length measurement.

Storage period after irradiation had no effect on germination. Germination percentage and sprout length of the seeds 1 day after irradiation or 5 months after irradiation had no significant difference (data was not shown).

3.2 Enzymatic activity for germination

The enzymatic activity in the germ of seeds can be analyzed

by Vita-Scope. As no life can exist without enzymatic activity, an evaluation of the enzymes in the germ will determine whether the germ is alive or not. So Vita-Scope measured the latent germination capacity. The result of enzymatic activity measurement was expressed as percentage of alive seeds. Figures 2 and 3 show the results of new seeds and old seeds, respectively. Enzymatic activity was inhibited at high dose irradiation. The difference between control and treatment was found at 1 kGy as shown in Table 3. However, some times, the difference could not be detected. The difference at 10 kGy was obvious and it could be detected all the time. No enzymatic activity existed in irradiated seeds at 100 kGy.

Enzymatic activity in old seeds was lower than that in new seeds. Storage after irradiation did not affect enzymatic activity and the similar results were obtained in 5 measurements. The Vita-Scope result of 10 kGy showed 41% of the seeds had enzymatic activity, whereas only 1 or 2 of 50 seeds sprouted with very short roots in germination test and they could not grow up. The results in Vita-Scope and germination tests suggested that the ability to germinate had been damaged, but the enzymatic activity still remained.

Compared with germination test, Vita-Scope is useful to get results quickly, but not as sensitive as germination. The enzymatic activity was partly inhibited in new seeds and nearly full inhibited in old seeds at 10 kGy. Based on the results of this experiment, Vita-Scope can detect 10 kGy irradiated seeds.

3.3 Amino acids analysis

The extent of losses of nutrients due to the irradiation of foods depends on many factors, such as the composition of food, the radiation dose, the temperature and the presence or absence of oxygen during irradiation. Usually, the protein content and the total nitrogen content in irradiated food were little changed. The change in amino acids composition of protein was reported (4,5).

Six ungerminated samples and two germinated samples were analyzed, respectively. The results were evaluated by double factors variation analysis. Table 4 shows the results of ungerminated seeds. Cystine and tryptophan were not included in this table because they were damaged by hydrolysis. The content of glycine, lysine and arginine was decreased at 10 kGy by 3.4, 5.2 and 5.1 %, respectively, but the content of valine, phenylalanine and arginine was increased by 4.0, 3.3 and 2.6% at 0.1 kGy ($p<0.05$), respectively. The content of some amino acids had a decline tendency as the dose increased, such as glycine, alanine, leucine, isoleucine, lysine, histidine and arginine.

It was found that the SD of methionine in Table 4 was larger than other amino acids. It was known that cystine which is a sulfer-containing amino acid was easily damaged by hydrolysis. It was possible that methionine, another sulfer-containing amino acid, was affected during hydrolysis because among these amino acids only methionine varied a lot. It was reported that the losses of amino acids in 5 kGy irradiated wheat seeds ranged from

10 to 20% (4). In this experiment, the change of glycine, lysine and arginine of which the difference was significant was about 5% in 10 kGy irradiated seeds.

Table 5 shows the mean value of two germinated samples. The content of lysine in irradiated seeds was less than in control. It was decreased by 23% at 10 kGy. Compared with ungerminated seeds, the difference between control and 10 kGy irradiated seeds was increased. The content in 100 Gy group was 9% higher than that in control. This difference might be caused by germination because the growth in 100 Gy is slightly better than that in control.

The results of both ungerminated and germinated seeds show that the effect of irradiation was small. The change in the content of lysine can be detected. For the purpose of detection of irradiated food, very careful operation and parallel samples and even repeat test were indispensable.

3.4 Measurement of chemiluminescence intensity

Chemiluminescence is the emission of light during chemical reaction. It is mostly produced by radicals. When the radicals from high energy position return to low energy position, red light will be released. CL intensity was corresponded to the amount of radicals. This technique has been used to detect irradiated spices, milk powder, onion and frozen chicken (6).

Figure 4 shows the change of CL intensity measured in dry condition of 10 kGy irradiated seeds during the first week after irradiation. CL intensity was strong after irradiation, but only

a few days later it decreased to the level of control. The effect of storage on CL intensity depends on the type of radicals. The CL intensity in Fig. 4 may be produced by short life radicals, which were not suitable to use them to identify irradiated seeds in dry condition, because the intensity disappeared quickly.

To see the effect of germination on CL intensity, distilled water, 10 mM KCN solution and 2.5 mM gibberellic acid (GA) solution in 0.3% ethanol were used. KCN can increase CL intensity during soybean germination (7). GA was a hormone for plant growth. The CL intensity curves are shown in Fig. 5. CL intensity was increasing sharply immediately after the addition of solution, and then decreasing gradually. However, no obvious change of CL intensity appeared during germination. The CL intensity change was also observed at 8, 16 and 24 h. The results were similar to that of 5 h.

Although these results suggest that no change of CL intensity occurred during germination, the CL intensity was different at different dose. To observe the effect of 3 months storage on CL intensity, the solution of one part ethanol in 5 parts distilled water was used instead of water to avoid seeds floating. Meanwhile, the temperature of measurement was changed into 45°C. At the first 2 or 3 min after liquid addition the intensity increased sharply and then leveled off (Fig. 6). The CL intensity was increased with the dose increasing. The intensity was gradually decreased during storage reducing the difference between the samples irradiated and unirradiated. The

CL intensity of the mean value of 12 min observation is shown as count/sec in Table 6. The result shows that the CL intensity increased with the dose and decreased during the storage. The significance ($p<0.01$) was found at 10 kGy. And the results of 15 days and 1 month were higher than others. Moisture content may affect CL intensity. Seed dried at 105°C for 4 h in advance much more increased CL intensity.

According to this experiment, no obvious CL intensity occurred during germination. The CL intensity may be produced by long life radicals. The change at 10 kGy can be detected for 3 months after irradiation. As the value of control was decreased during storage, it became difficult to identify low dose irradiated seeds.

3.5 Measurement of electron spin resonance spectrum

ESR technique has been proposed as a method for identification of irradiated food (3). ESR signal in barley irradiated at 10 kGy can be detected (8). In this experiment, skinned wheat seeds were used. Two kinds of radicals were detected in irradiated seeds by ESR analysis. The g values of them were 2.0055 and 2.0063 (Fig. 7). The former might be quinone radical whose intensity was in proportion to irradiation dose (Fig. 8). The later might be peroxide radicals whose intensity was not in relation to irradiation dose. Figure 8 shows a good linear relation existed between ESR intensity and the logarithm of dose. It is known that ESR signal intensity increased as radiation dose increased, and decreased with storage

time (9). The ESR signal in black and white peppers induced by irradiation at 10 kGy can be identified after 13 weeks (10).

The results suggested that these two kinds of radicals were not in relation with germination. Although the single intensity was in proportion to radiation dose, storage effect on the decay of radicals was not analyzed in this experiment. Further research should be continued to evaluate the sensitivity of detecting irradiated seeds by ESR spectrum measurement.

4. CONCLUSION

In this research, 5 methods were tested to detect components change in irradiated seeds for identification of irradiated food. All the 5 methods can be used to identify irradiated seeds, but the sensitivity was different.

(1) The seeds irradiated at 500 Gy can be identified by germination. Storage did not affect detection.

(2) Vita-Scope test based on the enzymatic activity for germination was not so sensitive as germination. It can identify 10 kGy irradiated seeds in 4 months after irradiation.

(3) The decrease of the content of lysine at 10 kGy can be detected by amino acids analysis, but the change was small.

(4) The CL intensity increased with radiation dose and decreased during storage. The seeds irradiated at 10 kGy can be identified after 3 months storage.

(5) ESR analysis is sensitive to radiation. The increase of ESR

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signal intensity at 100 Gy can be detected after irradiation.

Among these methods, germination is the most sensitive and was not affected by storage. The sensitivity of 500 Gy is suitable for identification of irradiated wheat in commercial use.

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REFERENCES

1. FAO/IAEA/WHO: Wholesomeness of Irradiated Food (Summary of the Report of the Joint FAO/IAEA/WHO Expert Committee, WHO Technical Report Series No. 659 (1981).

2. P. Loaharanu: Worldwide status of food irradiation and the FAO/IAEA/WHO/ITC-UNCTAD/GATT international conference on the acceptance; control of and trade in irradiated food, Radiat. Phys. Chem., 34, 1013 (1989).

3. H. Delincee and D.A.E. Ehlermann: Recent advances in the identification of irradiated food, Radiat. Phys. Chem., 34, 877-890 (1989).

4. A.B. Khattak and C.F. Klopfenstein: Effect of gamma irradiation on the nutritional quality of grins and legumes. II. Changes in amino acid profiles and available lysine, Cereal Chem., 66, 171-172 (1989).

5. E.Z. Naji, H. Jaddou, and A.M. Siddiqi: Effect of γ-irradiation on protein and amino acid compositions of broad beans, FSTA, 20, No.12 (1988).

6. W. Bogl and L. Heide: Chemiluminescence measurements as an identification method for gamma-irradiated foodstuffs, Radiat. Phys. Chem., 25, 173-185 (1985).

7. R. Saeki, K. Hishinuma, M. Usa, N. Watanabe, S. Mashiko, M. Kobayashi, T. Ando, K. Ogawa, T. Ichimura, B. Yoda & H. Inaba: Proceedings of Japan laser medicine, 8, 95-96 (1987).

8. J. Raffi, J.P. Agnel and S.R. Kassis: Identification par resonance paramagnetique electronique de cereales irradiees, Sci. Aliments, 7, 657 (1987).

9. M.H. Stevenson and R. Gray: Effect of irradiation dose storage time and temperature on the ESR signal in irradiated chicken bone, J. Sci. Food Agric., 48, 269-274 (1989).

10. N. Morishita, T. Kume, W. Kawakami and I. Ishigaki: Identification of irradiated pepper by ESR measurement, Food Irradiation, Japan, 23, (1988).

Table 1 Effect of irradiation on wheat seed sprouting*

Dose (kGy)	No. of sample	Germination (%)	Sprout** length(mm)	Root** length(mm)
Control	50	84	67±9	62±10
0.1	50	80	67±8	63±13
0.33	50	76	50±12[b]	35±10[b]
0.66	50	78	20±2[b]	11±3[b]
1	50	78	16±4[b]	8±4[b]
3.3	50	60[b]	----	----
6.6	50	32[b]	----	----
10	50	2[b]	----	----

*: Germination was defined as the protrusion of sprout and root to the extent of at least 3 mm length.

**: Mean±SD of the first 60% of the seeds which had longest sprout and root.

b: Compared with control, $p<0.01$.

----: Not measured.

Table 2 Effect of low dose irradiation on seed germination and growth

Irradiation condition	Dose (Gy)	Germination (%)		Sprout length (mm)*	
		new seed	old seed	new seed	old seed
Air	Control	80	72	67±5	46±13
	1	84	62	72±4	40±18
	10	76	68	72±4	45±14
	100	82	82	70±6	47±8
	500	72	84	18±2[b]	19±4[b]
O_2	Control	92	84	61±7	51±7
	1	88	80	72±9	62±6
	10	80	72	66±9	60±8
	100	84	84	57±6	57±10
	500	88	84	14±2[b]	17±4[b]
N_2	Control	80	68	62±7	52±10
	1	92	80	65±7	56±8
	10	80	80	66±10	58±11
	100	88	72	69±6[c]	56±9
	1000	84	68	12±2[b]	12±2[b]

*: Mean±SD

b: Compared with control in the same condition, the decrease was significant, $p<0.01$.

c: Compared with control in the same condition, the increase was significant, $p<0.05$.

Table 3 Percentage of alive seed during storage

Time after irradiation	Alive seed (%)			
	Control	0.1 kGy	1 kGy	10 kGy
New seed				
1 day	96	92	84[a]	50[b]
7 days	96	86	82[a]	44[b]
1 month	98	88	80[a]	20[b]
2 months	98	98	82[a]	44[b]
4 months	98	96	94	48[b]
Mean±SD	97±1	92±5	84±6[b]	41±12[b]
Old seed				
1 day	70	52	52	16[b]
7 days	58	48	44	10[b]
1 month	62	44	34[b]	0[b]
2 months	66	54	44[a]	2[b]
4 months	76	60	52[a]	8[b]
Mean±SD	66±7	52±6[a]	45±7[b]	7±6[b]

a,b: Compared with control in the same condition, the decrease was significant, $p<0.05$ and $p<0.01$, respectively.

Table 4 Effect of gamma irradiation on amino acid composition of wheat seed (nmol/injection)*

Amino acid	Control	0.1 kGy	1 kGy	10 kGy
THR	11.91±0.38	12.19±0.13	11.99±0.34	12.01±0.43
SER	18.48±0.81	19.12±0.46	18.36±0.73	18.40±0.44
GLY	29.31±0.90	29.62±0.43	28.75±0.75	28.75±0.74[a]
ALA	10.64±0.34	10.68±0.23	10.48±0.29	10.29±0.29
VAL	16.72±0.73	17.39±0.64[a]	17.03±0.98	16.44±0.35
MET	4.48±0.41	3.27±1.88	4.74±0.71	4.01±1.26
ILEU	12.51±0.54	12.79±0.22	12.66±0.59	12.38±0.20
LEU	23.84±0.79	24.35±0.39	23.92±0.85	23.71±0.46
TYR	5.51±0.78	5.36±0.31	5.48±0.60	4.99±0.58
PHE	12.57±0.29	12.99±0.15[a]	12.54±0.38	12.57±0.24
LYS	6.71±0.16	6.68±0.19	6.63±0.19	6.36±0.10[b]
HIS	8.03±0.23	8.21±0.10	7.97±0.26	7.86±0.22
ARG	17.03±0.38	17.48±0.27[a]	16.87±0.43	16.16±0.29[b]

a,b: Compared with control, the difference was significant, $p<0.05$ and $p<0.01$, respectively.

*: Mean±SD of six samples.

Table 5 Content of amino acids of irradiated and germinated wheat seed (nmol/injection)*

Amino acid	Control	0.1 kGy	1 kGy	10 kGy
THR	13.44	14.14	12.26	13.10
SER	16.97	19.14	17.02	18.78
GLY	29.31	31.93	28.25	30.10
ALA	26.79	28.36	25.68	23.60
VAL	18.30	19.96	17.18	18.41
MET	4.48	4.76	4.34	4.76
ILEU	13.54	14.76	12.60	13.66
LEU	24.36	26.92	23.37	25.68
TYR	7.02	8.30	7.26	7.50
PHE	12.22	13.80	11.86	13.40
LYS	9.30	9.22	7.51[b]	7.14[b]
HIS	7.81	8.61	7.86	8.18
ARG	17.00	18.24	16.00	16.74

*: Mean of two samples.
b: $p<0.01$

Table 6 Effect of irradiation and storage period on chemiluminescence intensity (count/sec)*

	Control	0.1 kGy	1 kGy	10 kGy
15 days	254	276 (22)[a]	283 (29)[b]	425 (171)[b]
1 month	244	256 (12)	280 (36)[b]	371 (127)[b]
2 months	207	231 (24)[a]	248 (41)[b]	326 (119)[b]
3 months	224	232 (8)	244 (20)	289 (65)[b]

*: The difference between treatment and control in parentheses.
a,b: Compared with control, the difference was significant, $p<0.05$ and $p<0.01$, respectively.

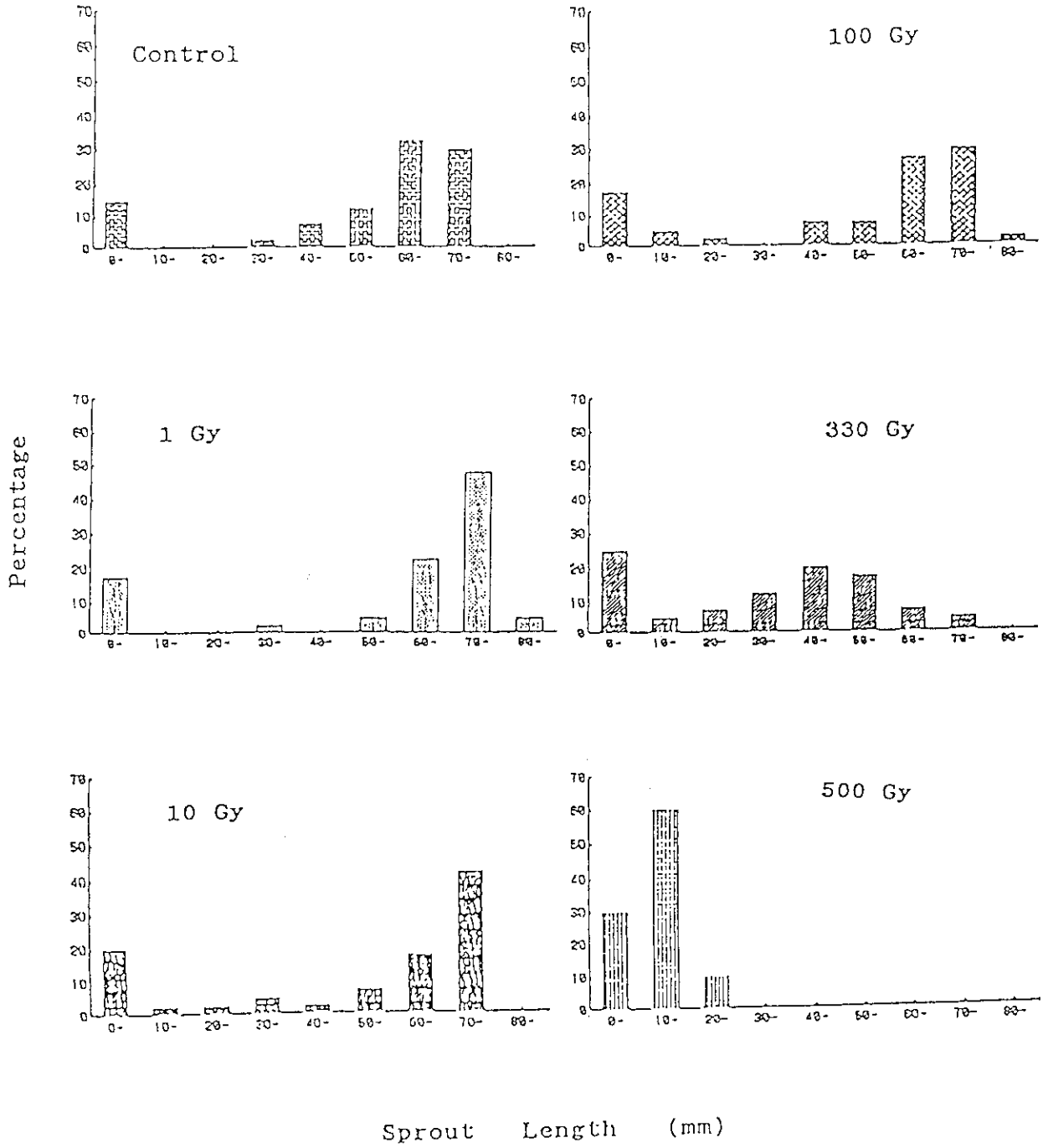

Fig. 1 Sprout length of irradiated wheat seeds after 5 days germination

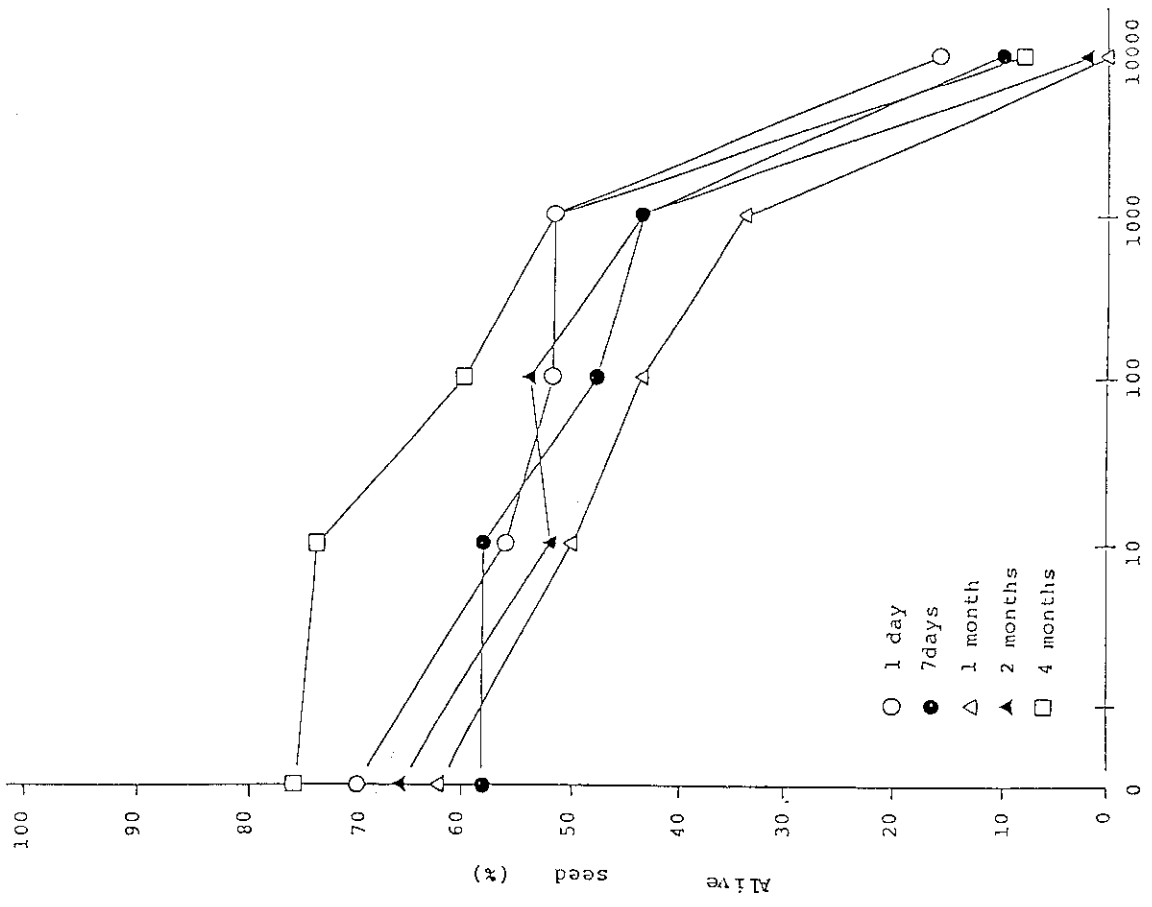

Fig. 2 Effect of irradiation and storage on enzymatic activity for germination of new seeds

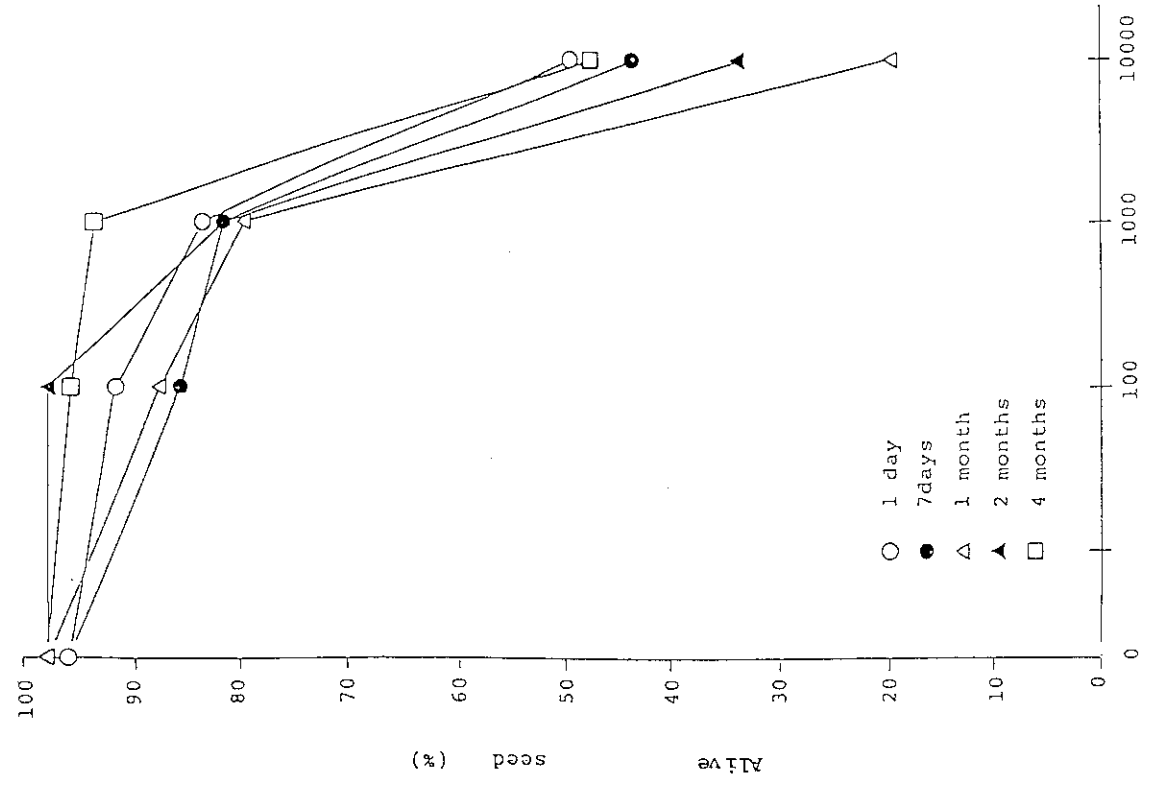

Fig. 3 Effect of irradiation and storage on enzymatic activity for germination of old seeds

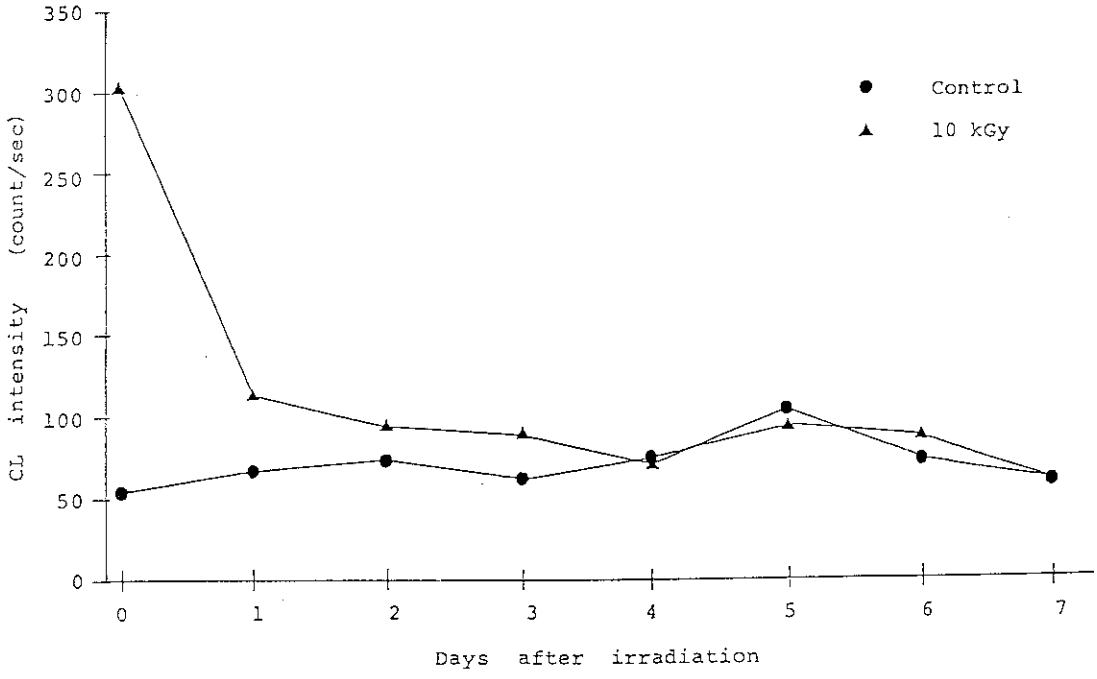

Fig. 4 Effect of storage time on CL intensity of dry seed

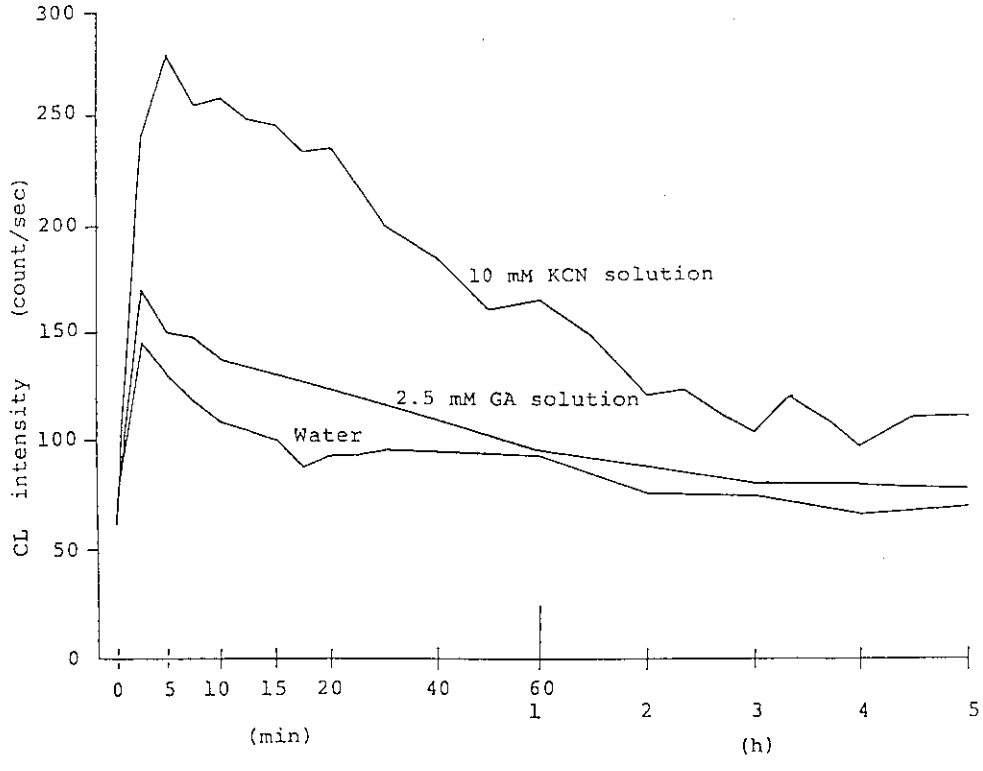

Fig. 5 The change of CL intensity during germination of control seed in different solution

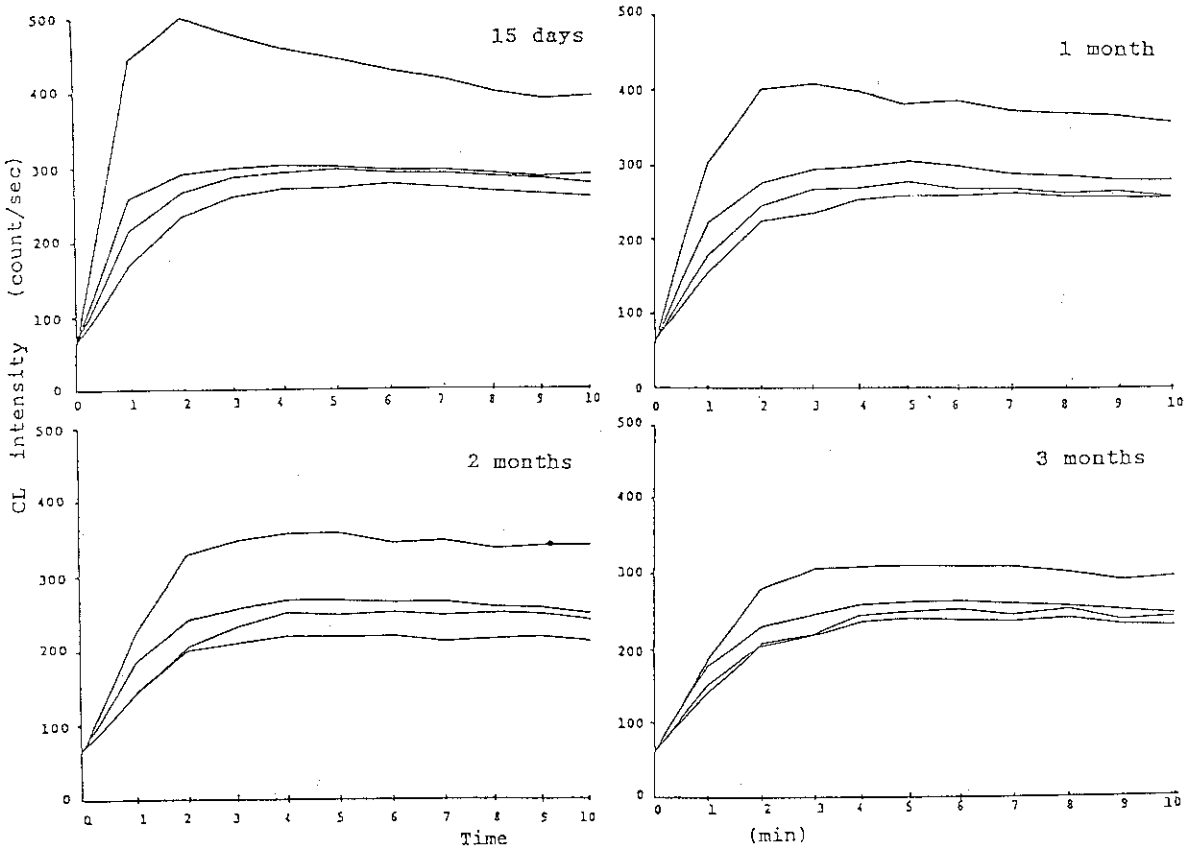

Fig. 6 Effect of irradiation and storage on CL intensity of wheat seed

The curves from top to bottom were 10000, 1000, 100 Gy and control, respectively.

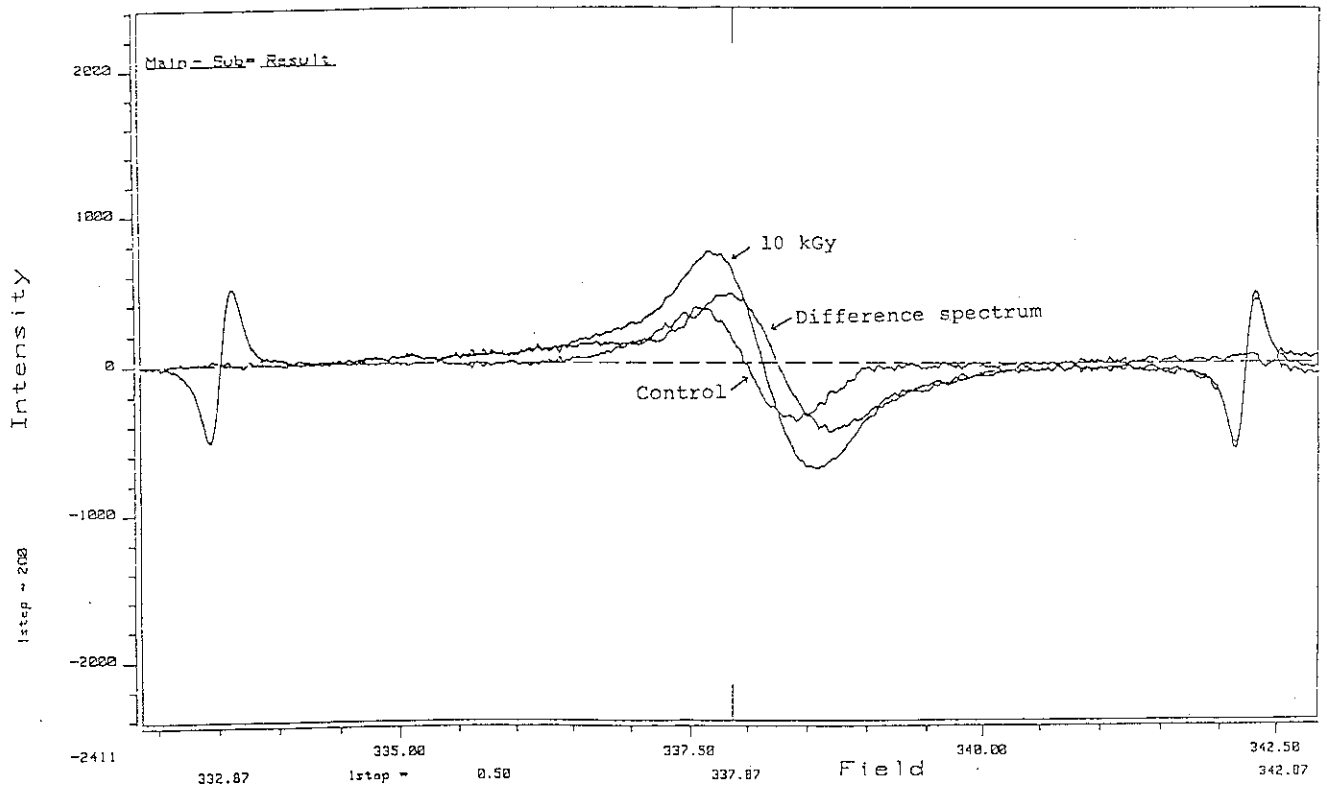

Fig. 7 The ESR spectrum of irradiated seed

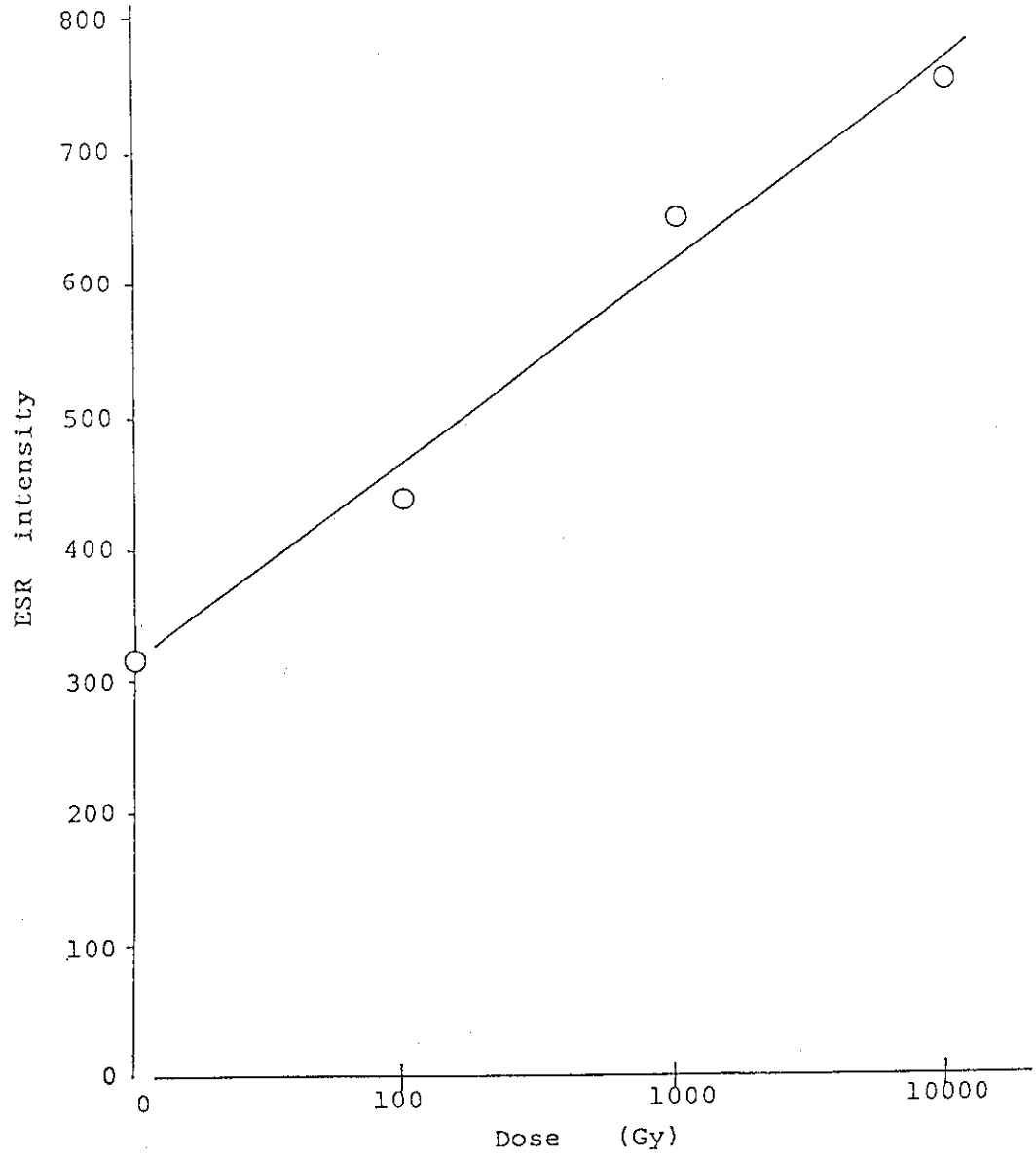

Fig. 8 Effect of radiation dose on ESR intensity