# Radiobiological Studies on Plants. III

## Sensitivity of the Seeds of Wheats and Oats to Gamma-Rays from $Co^{60}$*

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Comparisons among various ionizing radiations of the direct effects and genetic disturbances to plants are now in progress at the Laboratory of Genetics. The present paper is concerned with the effects of gamma-rays. The plants used for the experiments were wheats and oats. The object of the experiment was to know if there were any differences of the effects of gamma-rays according to the species of plants or polyploidy. Sensitivity of the plants was expressed by germination rate, survival rate, development of the plant organs above- and under-ground, and the degrees of developmental disturbances to the first and the third leaves. The results of the experiments are summarized as follows:

1. Germination rate of the dry seeds of wheats and oats irradiated by dosages up to 70 kr of gamma-rays was usually from 80% to 100% or 70–75% in a few cases and was practically the same as that of the control.
2. Survival rate of the plants irradiated by the same dosage was not the same between wheats and oats. It also varied according to the species within the genus. It can safely be said in some cases that the higher the degrees of polyploidy the lower was the sensitivity to gamma-rays.
3. Comparisons of the effects of gamma-rays between the above-ground and under-ground parts of plants showed that the degrees of disturbances were the same at both parts in the various plants and at the varying dosages of gamma-rays.
4. The leaves which developed from the irradiated seeds were characteristic of shorter and narrower leaves. Comparisons of disturbances due to gamma-rays between the first and third leaf, taking the leaf length and width as the standard of disturbances, show that the degrees of disturbances were much greater to the third leaves than to the first leaves in various plants and at various dosages administered.

## INTRODUCTION

The direct disturbances and genetic effects to plants of fast neutron, gamma-rays from $Co^{60}$, X-rays and resonance neutron are being studied. In the present experiment dry seeds were irradiated by gamma-rays and direct disturbances of the plant growth at the initial stage were studied.

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The plants used for the experiment were wheats and oats.

The aim of the present experiment was to see if there were any differences of gamma-rays according to the species or polyploidy of plants. As standards of sensitivity to gamma-rays, germination rate, survival rate, comparisons of disturbances between above-ground and under-ground parts of plants and comparisons of disturbances between the first leaves and the third leaves were employed.

Studies on the effects of gamma-rays from $Co^{60}$ on the higher plants such as barley, wheat, bean and potato have been reported by Sparrow and Christensen (1950), Ehrenberg, Granhall, Gustafsson and Nybom (1954), Nybom, Gustafsson, Granhall and Ehrenberg (1956) and Matsumura and Fujii (1957). The present report is, in certain respects, the same as the above reports except the materials used. But special emphasis will be placed on the relation between polyploidy and sensitivity, and on the relation between the stages of development of organs and disturbances inflicted to plants by gamma-rays from $Co^{60}$.

## MATERIALS AND METHODS

The materials used were dry seeds of four species including five varieties of wheats : *Triticum monococcum* var. *vulgare* Körn. (2*x*), *T. dicoccum* Schübl. (4*x*). *T. persicum* L. (4*x*), *T. vulgare* Vill. var. Shinchunaga (6*x*), *T. vulgare* Vill. var. Igachikugo (6*x*) and three species including five varieties of oats : *Avena strigosa* Schreb. (2*x*), *A. strigosa* Schreb. (4*x*, autotetraploid), *A. barbata* Pott (4*x*), *A. sativa* L. (6*x*), *A. sativa* L. var. Kanota (6*x*). The seeds used for the experiment were of uniform size and weight selected from those harvested in 1958. Sixty seeds were used in each class. Irradiation was done at the gamma-ray irradiation facility at the Institute for Chemical Research, Kyoto University, the dosage being 5kr, 10kr, 20kr, 30kr, 40kr, 50kr, 60kr, and 70kr.

Germination rate of seeds was estimated by counting the number of seeds germinated on petri-dishes in which two sheets of filter paper were placed in order to maintain uniform supply of water.

Survival rate and disturbances to the first and third leaves were calculated with the plants which grew in boxes filled with burnt soil.

At the comparisons of the effects between above-ground and under-ground parts, the seeds were sown in boxes filled with sand. Special cares were taken so as not to injure roots when the plants were pulled off the sand at measurement.

## EXPERIMENTAL RESULTS

### 1. Germination Rate

Both control and irradiated seeds were sown at the same time immediately after irradiation. The seeds whose seedlings and seminal roots were 0.3 cm or more were counted as germinated, very little differences of germination rate of seeds among ten species or varieties used for the experiment were observed between the control and irradiated seeds within the dosages from 5kr to 70kr.

That is to say, germination rate was usually more than 80% in all experiments with a few cases showing 70–75% germination in both of the control and irradiated seeds. However, the time required from sowing to germination was about 24 hours or more with *A. sativa* seeds irradiated with high dosages above 50kr and with the other seeds irradiated by dosages above 30kr or 40kr. No. abnormalities of germination were noticed with dry seeds left for about three months after irradiation.

## 2. Survival Rate

As a standard for sensitivity of seeds to radiations, LD-50 has frequently been adopted. In order to find LD-50 of wheat and oat seeds at ten days after sowing and at maturity, the development, particularly the survival rate of the seeds irradiated by dosages from 5 kr to 70 kr was investigated.

Table 1 shows the survival rate at ten days after sowing of the seeds sown immediately after irradiation. Table 2 shows LD-50 obtained from Table 1 at ten days after sowing. Survival rate at maturity is shown in Table 3, from which LD-50 listed in Table 2 was obtained.

When LD-50 is used as a standard of sensitivity of various plants to ionizing

Table 1. Survival rate at 10 days after sowing.

| Species | Cont. | 5 kr | 10 kr | 20 kr | 30 kr | 40 kr | 50 kr | 60 kr | 70 kr |
|---|---|---|---|---|---|---|---|---|---|
| *T. monococcum* | 80.5 | 82.4 | 87.6 | 58.1 | 41.0 | 0 | 0 | 0 | 0 |
| *T. dicoccum* | 100.0 | 93.2 | 89.9 | 84.3 | 70.0 | 73.1 | 32.8 | 0 | 0 |
| *T. persicum* | 97.1 | 93.3 | 100.0 | 97.7 | 84.3 | 2.8 | 0 | 0 | 0 |
| *T. vulg.* (Shinchunaga) | 96.7 | 90.0 | 100.0 | 100.0 | 87.4 | 16.2 | 0 | 0 | 0 |
| *T. vulg.* (Igachikugo) | 96.7 | 100.0 | 93.3 | 100.0 | 83.5 | 80.0 | 6.4 | 0 | 0 |
| *A. strigosa* 2x | 100.0 | 100.0 | 100.0 | 91.6 | 83.5 | 43.8 | 0 | 0 | 0 |
| *A. strigosa* 4x | 93.3 | 86.7 | 93.3 | 90.0 | 90.0 | 83.3 | 46.7 | 0 | 0 |
| *A. barbata* | 86.7 | 83.3 | 92.3 | 72.3 | 81.4 | 68.0 | 10.2 | 0 | 0 |
| *A. sativa* | 100.0 | 96.8 | 100.0 | 93.3 | 97.8 | 90.3 | 87.8 | 76.2 | 43.3 |
| *A. sativa* (Kanota) | 97.8 | 96.8 | 96.8 | 95.3 | 89.3 | 100.0 | 81.3 | 55.4 | 40.1 |

Table 2. Dosages (kr) of LD-50 at 10 days after sowing and at maturity.

| Species | At 10 days after sowing | At maturity |
|---|---|---|
| *T. monococcum* | 20 | 10–20 |
| *T. dicoccum* | 40–50 | 20–30 |
| *T. persicum* | 30–40 | 20–30 |
| *T. vulg.* (Shinchunaga) | 30–40 | 20–30 |
| *T. vulg.* (Igachikugo) | 40–50 | 20–30 |
| *A. strigosa* 2x | 40 | 20 |
| *A. strigosa* 4x | 50 | 20–30 |
| *A. barbata* | 40 | 20 |
| *A. sativa* | 60–70 | 30 |
| *A. sativa* (Kanota) | 60 | 30–40 |

Table 3. Survival rate at maturity.

| Species | Cont. | 5 kr | 10 kr | 20 kr | 30 kr | 40 kr |
|---|---|---|---|---|---|---|
| *T. monococcum* | 85.6 | 87.7 | 72.5 | 0 | 0 | 0 |
| *T. dicoccum* | 93.5 | 98.2 | 89.1 | 68.3 | 10.2 | 0 |
| *T. persicum* | 93.4 | 89.8 | 80.2 | 82.3 | 0 | 0 |
| *T. vulg.* (Shinchunaga) | 98.0 | 93.5 | 92.1 | 79.5 | 0 | 0 |
| *T. vulg.* (Igachikugo) | 95.8 | 96.5 | 91.5 | 80.2 | 0 | 0 |
| *A. strigosa* 2x | 98.3 | 100.0 | 81.3 | 58.4 | 0 | 0 |
| *A. strigosa* 4x | 84.5 | 86.6 | 81.5 | 72.5 | 13.5 | 0 |
| *A. barbata* | 100.0 | 100.0 | 85.3 | 59.2 | 0 | 0 |
| *A. sativa* | 95.4 | 92.7 | 100.0 | 91.8 | 57.3 | 0 |
| *A. sativa* (Kanota) | 100.0 | 98.5 | 93.5 | 92.8 | 69.1 | 0 |

radiations, it is noticed that resistance of oats against ionizing radiations is stronger than that of wheats and that in some cases resistance clearly increases corresponding to the degree of polyploidy. A good example of this is that the autotetraploid of *Avena strigosa* is more resistant to ionizing radiations than the diploid species.

It is naturally to be expected that the dosage of LD-50 becomes less with the growth of plants, because plants which escaped death at the earlier stages of growth might, in some cases, be destroyed at the later stages of growth.

### 3. Comparisons of Effects to Above-ground and Under-ground Parts of Plants

Numerous results have been published on the direct disturbances inflicted to above-ground parts of plants by ionizing radiations. The authors intended to clarify the developmental disturbances of above-ground parts as well as under-ground parts, roots, of plants. Plant height was adopted as a standard of disturbances to above-ground parts and length of root as that for under-ground

Table 4 Comparisons of the disturbances to the above-ground and under-ground parts of plants.

Table 4-1. 17 days after sowing in *T. vulg.* Igachikugo.

| | Control | 10 kr | 20 kr | 30 kr |
|---|---|---|---|---|
| Above-ground | 100.0 (10.4) | 89.3 ( 9.2) | 68.0 (7.0) | 30.1 (3.1) |
| Under-ground | 100.0 (14.5) | 84.1 (12.2) | 55.9 (8.1) | 23.4 (3.4) |

Table 4-2. 10 days after sowing in *T. vulg.* Shinchunaga.

| | Control | 30 kr | 40 kr | 50 kr |
|---|---|---|---|---|
| Above-ground | 100.0 (11.0) | 40.5 (4.5) | 26.1 (2.9) | 16.2 (1.8) |
| Under-ground | 100.0 (12.5) | 48.8 (6.1) | 29.6 (3.7) | 19.1 (2.2) |

Table 4-3. 15 days after sowing in *T. vulg.* Shinchunaga.

| | Control | 30 kr | 40 kr | 50 kr | 60 kr |
|---|---|---|---|---|---|
| Above-ground | 100.0 (15.8) | 51.3 (8.1) | 29.7 (4.7) | 15.8 (2.5) | 11.4 (1.8) |
| Under-ground | 100.0 (15.6) | 55.7 (8.7) | 31.4 (4.9) | 16.7 (2.6) | 12.2 (1.9) |

Table 4-4. 17 days after sowing in *A. strigosa* 2*x*.

| | Control | 10 kr | 20 kr | 30 kr |
|---|---|---|---|---|
| Above-ground | 100.0 ( 7.6) | 93.4 (7.1) | 60.5 (4.6) | 32.9 (2.5) |
| Under-ground | 100.0 (10.5) | 85.7 (9.0) | 53.3 (5.6) | 39.0 (4.1) |

Table 4-5. 10 days after sowing in *A. sativa.*

| | Control | 30 kr | 40 kr | 50 kr | 60 kr |
|---|---|---|---|---|---|
| Above-ground | 100.0 ( 8.8) | 59.1 (5.2) | 36.4 (3.2) | 20.5 (1.8) | 15.9 (1.4) |
| Under-ground | 100.0 (12.2) | 41.0 (5.0) | 21.3 (2.6) | 12.3 (1.5) | 10.0 (1.2) |

Table 4-6. 20 days after sowing in *A. sative.*

| | Control | 30 kr | 40 kr | 50 kr |
|---|---|---|---|---|
| Above-ground | 100.0 (12.3) | 70.7 ( 8.7) | 38.2 (4.7) | 40.7 (5.0) |
| Under-ground | 100.0 (19.6) | 66.3 (13.0) | 22.0 (4.3) | 23.0 (4.4) |

The number in parenthesis shows actual length in cm.

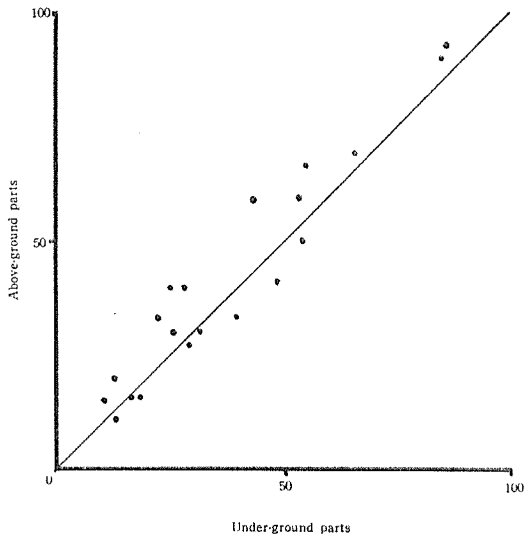


Fig. 1. Correspondence of the effects of gamma-rays to under-ground parts to that to above-ground parts of plants from irradiated seeds.

parts. In Tables 4-1~6, the degrees of disturbances are denoted by decrease of indices in the irradiated classes, plant, height and root length in the control being taken as 100.

According to the tables, the indices of the above-ground parts and those of the corresponding under-ground parts are almost equal in the various plants, in various species within the same genus at varying dosages. Fig. 1 is the graphic representation of the results in the Tables 4-1~6. The Y axis shows the indices of above-ground parts and the X axis those of under-ground parts. The diagonal is the line where the indices of X and Y are equal. The values obtained in the experiment are almost in all cases congregated close to this diagonal. On this basis it may safely be asserted that primodial tissues or cells of young seedlings and those of roots are almost equally sensitive to ionizing radiations.

### 4. Effect of Gamma-Rays on Development of the First Leaf and the Third Leaf

Compared with the control plants, the leaf-blades of the plants which develop from irradiated seeds are shorter and narrower. It was noticed that these effects vary with the leaves of the different leaf order, which made us to undergo detailed investigation on comparisons of direct effects with the first and the third leaves. Both leaf length and width were measured at the stage where

Table 5. Indices showing length of leaf-blade.

| Species | Leaf order | Control | 30 kr | 40 kr | 50 kr | 60 kr |
|---|---|---|---|---|---|---|
| *T. dicoccum* | 1st Leaf | 100.0 | 63.4 | 50.8 | 39.6 | 56.0 |
| | 3rd Leaf | 100.0 | 59.4 | 47.4 | 30.8 | 41.3 |
| *T. persicum* | 1st Leaf | 100.0 | 58.6 | 49.0 | | |
| | 3rd Leaf | 100.0 | 54.9 | 37.1 | | |
| *T. vulg.* Shinchunaga | 1st Leaf | 100.0 | 44.8 | 30.2 | | |
| | 3rd Leaf | 100.0 | 53.8 | 18.1 | | |
| *T. vulg.* Igachikugo | 1st Leaf | 100.0 | 50.7 | 23.0 | | |
| | 3rd Leaf | 100.0 | 71.6 | 23.3 | | |

Table 6. Indices showing width of leaf-blade.

| Species | Leaf order | Control | 30 kr | 40 kr | 50 kr | 60 kr |
|---|---|---|---|---|---|---|
| *T. dicoccum* | 1st Leaf | 100.0 | 68.8 | 63.1 | 64.2 | 37.6 |
| | 3rd Leaf | 100.0 | 45.9 | 28.4 | 35.4 | 32.4 |
| *T. persicum* | 1st Leaf | 100.0 | 74.7 | 76.9 | | |
| | 3rd Leaf | 100.0 | 47.4 | 34.2 | | |
| *T. vulg.* Shinchunaga | 1st Leaf | 100.0 | 63.2 | 55.6 | | |
| | 3rd Leaf | 100.0 | 61.8 | 22.9 | | |
| *T. vulg.* Igachikugo | 1st Leaf | 100.0 | 72.5 | 42.5 | | |
| | 3rd Leaf | 100.0 | 47.2 | 27.0 | | |

Table 7. Size indices (width×length) of leaf-blade.

| Species | Leaf order | Control | 30 kr | 40 kr |
|---|---|---|---|---|
| *T. dicoccum* | 1st Leaf | 100.0 | 30.6 | 17.8 |
| | 3rd Leaf | 100.0 | 22.4 | 3.1 |
| *T. persicum* | 1st Leaf | 100.0 | 43.7 | 21.7 |
| | 3rd Leaf | 100.0 | 27.3 | 8.7 |
| *T. vulg.* Shinchunaga | 1st Leaf | 100.0 | 38.6 | |
| | 3rd Leaf | 100.0 | 31.6 | |
| *T. vulg.* Igachikugo | 1st Leaf | 100.0 | 44.9 | |
| | 3rd Leaf | 100.0 | 26.8 | |

the leaves stopped elongation. The decrease of leaf-blade length and that of width in irradiated plants were expressed by the decrease of indices in irradiated plants as compared with control plants. Table 5 shows the indices of leaf-blade length in various plants. As can clearly be noticed in the table, the indices of the third leaves are smaller than those of the first leaves without an exception. The same situation applies to the indices of width in Table 6. In the other words, the third leaves are more sensitive to radiations than the first leaves. In order to make this point clearer, the indices of leaf-blade (length×width) were adopted in Table 7.

The fact that the disturbances to the third leaves are always greater than those to the first leaves in the various species and at various dosages of gamma-rays can be said to be due to the differences of radiation sensitivity to the developmental stages of organs. That is to say, the third leaves are less developed than the first leaves at the embryonic stage, which caused differences in the disturbances induced. Thus the less the degrees of differentiation of organs, the greater would be the direct effects of ionizing radiations.

## DISCUSSION

Very little differences of germination rate of the dry seeds irradiated by gamma-rays from $Co^{60}$ within the ranges of 5kr~70kr were observed. The germination rate was from 80% to 100%.

Entirely the same results were obtained with X-ray irradiation within the dosages ranging from 5 kr to 500 kr (Nishiyama unpublished). It was found that the same situation holds with seeds irradiated by ultra-high dosage of thermal neutron. The dry seeds of above ten materials which were irradiated by $2.3\times10^{15}N_{th}/cm^2$ dosage of neutron at Beam Hole No. 16 of JRR-I, showed very good germination rate without an exception. The irradiated seeds, which germinated, grew up to 0.3~1.0 cm and died later. However, the seeds irradiated by dosages above $4.6\times10^{16}N_{th}/cm^2$ completely lost the ability of germination (Nishiyama unpublished).

The above results are very much different from those obtained by MacKey (1951), Matsumura and Fujii (1957), and Fujii (1958a), who reported lowering of germination rate at the dosages of 30 kr or 40 kr.

As to survival rate, sensitivity varies with plants as are shown in Table 4. Generally speaking, wheats are more sensitive than oats. These differences of sensitivity to radiation have already been reported by Beard, Haskins and Gardner (1958) with barley, maize, mustard and safflower. It appears in some cases that the differences of sensitivity in various species within the same genus are closely related to polyploidy. That is to say, the higher the polyploidy, the greater will be the resistance. It is particularly interesting to find that between a diploid species, *A. strigosa* and its autotetraploid, the diploid is more sensitive than autotetraploid.

When sensitivity of the above-ground and under-ground parts of plants is measured in terms of plant height and elongation of root, the degrees of disturbances to leaves and roots are almost 1 : 1 in the 1) various plants, 2) at different stages of development and 3) at varying dosage of gamma-rays. This fact makes us to infer that the anlages of leaves and those of roots are equally sensitive to radiations. Almost the same results have been obtained in the disturbances to survival rate and to above-ground and under-ground parts at the experiment on the effects of X-rays and thermal neutron in the same plants as were used in the present experiment (Nishiyama, unpublished).

The plants irradiated by ionizing radiations are characteristic of shorter and narrower leaves. The degrees of these effects vary according to the leaf order. Detailed analysis showed that the third leaves were more strongly affected than the first leaves – the third leaves were shorter and narrower. Tables 5 and 6 show the indices of the various classes. According to the tables, indices of the third leaves were always smaller than those of the first leaves, in various plants and at various dosages of gamma-rays. This fact shows that the disturbances to the third leaves were greater than to the first leaves. This may be considered to suggest that the disturbances to organs vary according to the degrees of differentiation of the organs when the respective primodial tissues are irradiated.

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