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EFFECT OF NEUTRON AND GAMMA IRRADIATION ON THE GERMINATION OF DIPLOID AND TETRAPLOID RYE SEEDS

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任慶彬 : 中性子 및 감마線의 照射가 二倍體 및 四倍體 胡麥의
種子의 發芽 및 生長에 미치는 影響

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ABSTRACT

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Tetraploid rye, *Secale cereale* 4x, was more tolerant to fast nuetron than diploid rye. Root growth was more suppressed than was seedling height in both diploid and tetraploid rye. A stimmulative effect on the dry weight of the shoot could be observed at very low doses of irradiation. It was the fact that the lower the moisture content of the seeds, the higher the radiosensitivity. Concerning seedling height growth, the effectiveness ratio of N/X equalled about 20.0 in diploid rye and about 18.2 in tettraploid rye, when the 50% dose ratios is used for this quotient calculation.

INTRODUCTION

The dual purpose of this investigation was to study the importance of ploidy level in rye species for radiosensitivity and to compare the effectiveness of neutrons with that of gamma-rays. Several criteria, e.g., seedling height, germination percentage, root length growth and dry-matter content have been used to express effectiveness. The importance of different levels of water content for the radiosensitivity of seed was also investigated with respect to gamma irradiation.

The general effect of radiation on plant growth, the characteristics of ionizing radiation and the qualitative differnces between various kinds of radiation have previously been reviewed in several papers (Gray, 1946, Ehrenberg and Nybom, 1954, Ehrenberg, 1955, Ehrenberg, 1960).

MATERIALS AND METHODS

Two ploidy levels in rye species, *Secale cereale* 2x and 4x, were tested in this investigation. The rye seeds of the variety "Svalöf 0201" and the 4x rye seeds originated by chromosome doubling of the 2x-variety were both produced at the Swedish Seed Association and cultivated at its field station Ugerup in Southern Sweden.

After irradiation the seeds weeds were placed in petri-dishes (diameter, 8.5 cm) with two water retaining filter papers. All the petri-dishes were kept under continuous light exposure in a laboratory room where air-temperature was 22°±1. In one of the four replications the seeds were sown in sand under the same light and temperature conditions.

Neutron irradiation Neutron irradiation was applied at the atomic reactor, RI, in Stockholm by aid of Drs. L. Ehrenberg and G. Ahnström. The following neutron doses were given, 0, 280, 420, 560, 700, 840, 1120, 1400, 1680, 2100 and 2520 rads. Seeds were sown in petri-dishes (cf. above) 10 days after irradiation.

Gamma irradiation The gamma irradiation was applied at the Institute of Organic Chemistry and Biochemistry (Dr. L. Ehrenberg), University of Stockholm, where gamma-rays produced by a Co^{60} source giving a dose rate of 8100 r/min. The following doses were applied, 0, 8, 12, 18, 28, 42, 60, 80 and 120 k rads.

Before exposure the seeds were kept in desiccators with different relative air humidity. This was calibrated at various levels by forcing the air to pass through two vials containing aqueous KOH solutions of different concentrations (Kobayashi, 1932) before entering the desiccators where the seeds were stored for about two weeks. Immediately after irradiaton, the seeds were soaked in tap water in equilibration with air, and transferred to the petri-dishes. Water and light were continually supplied in all the series. The experiment complised thirty seeds in each treatment and two replications, e.g., a total of 2520 seeds for each ploidy level.

RESULTS AND DISCUSSION

Effect of neutron irradiation on rye seedlings A total of 960 seeds of each ploidy level were exposed, i. e., 30 seeds in each unit of treatment which was repeated four times. The shoot and the roots were measured eight days after sowing.

Data obtained have been presented in Fig. 1 and 2. All the the data are expressed in per cent of the measurements of untreated control plants. In the figure each vertical line indicates the two times of a standard deviation whereas dots indicate the mean values of four replications at each dose level. No

Fig. 1 EFFECT OF NEUTRON IRRADIATION OF DIPLOID AND TETRAPLOID RYE SEEDS ON SUBSEQUENT SEEDLING HIGHT GROWTH

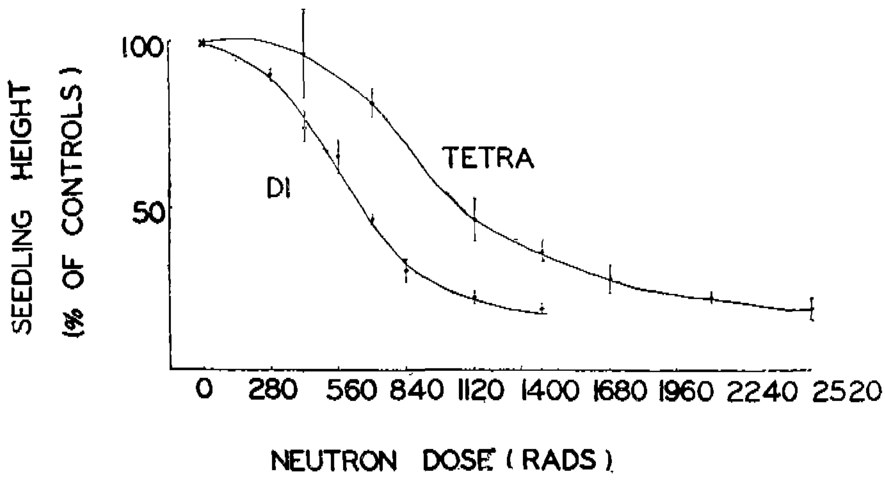
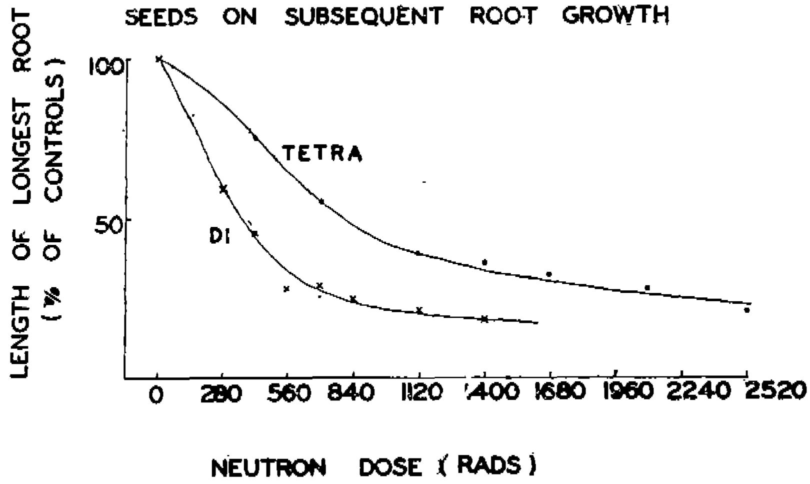


Fig. 2 EFFECT OF NEUTRON IRRADIATION OF DIPLOID AND TETRAPLOID RYE SEEDS ON SUBSEQUENT ROOT GROWTH



significant difference was found in this experiment between the length of shoot grown on filter paper and that of shoot on sand. The ratio of 50% neutron doses (50% suppression of growth comparing with control plants) between diploid rye and tetraploid rye approximately amounted to $\frac{1}{1.6}$ with respect to shoot growth. This value of the 50% dose ratio, is slightly higher than that of $\frac{1}{2.5}$ presented by Ehrenberg and Nybom (1954) for the effect of doubling chromosome number, as a modifying factor, on the sensitivity of seeds to neutron radiations.

Figure 1 shows that the range of variation of the four mean values for each dose level is narrower in diploid rye than that observed in tetraploid rye. The inhibition of root growth is presented in Fig, 2. The 50% dose ratio approximates $\frac{1}{2.4}$, e.g., a value rather equal to that presented by Ehrenberg and Nybom (1954). With respect to both shoot length and root growth, diploid rye is more sensitive to neutrons, relatively seen, than is tetraploid rye.

It should be pointed out, however, that diploid rye and tetraploid rye have appeared almost equally susceptible to radiation at doses exceeding 1680 rads. At this high doses, mostly coleoptile growth was measured. Some investigations (Ehrenberg and von Wettstein, 1955, Avanzi, 1960) indicate for neutron irradiated material that root growth is more suppressed than is shoot growth. A similar trend was also obtained in the present experiment for neutron doses below approx. 1120 rads in diploid rye and 1400 rads in tetraploid rye, i.e., more shoot growth in per cent to control and less root growth. After exposure to doses above this level, shoot growth was suppressed more than was root growth. This may be interpreted to imply that the shoot and the root differ with respect to radiation sensitivity when the neutron dose is increased.

The germinability was not affected by differences in neutron doses in both diploid rye and in tetraploid rye. Even exposure to 2520 rads produced normal rates of germination.

Some stimulation of dry weight as expressed by increase in shoot growth could be observed particularly in tetraploid rye, at doses between 280 rads and 700 rads.

Effect of gamma irradiation on rye seedlings After moisture equilibration, the following levels of seed moisture were recorded (Table 1).

Table 1. Computation of moisture content on the basis of weight (60 seeds in each treatment).

Solution component	Moisture content per cent	
	diploid	tetraploid
H_2SO_4 95%	6.91	7.28
100 g. KOH+100 ml. H_2O	10.82	10.67
70 〃 〃	12.63	12.61
55 〃 〃	14.37	14.15
40 〃 〃	16.32	16.23
20 〃 〃	16.77	16.68
5 〃 〃	28.00	28.60

After irradiation the seeds were sown and ten days later the length and oven-dry weight of the shoot were determined. The primordial leaf developing from the coleoptile in tetraploid rye seemed to grow faster than that in diploid rye. Gamma-ray doses exceeding 40 K rads and 60 K rads in diploid and tetraploid rye, respectively, produced equal degree of suppression of shoot growth, although the variation of the water content of the seeds was great. At a dosage of 28 K rads, diploid rye suddenly appeared to be damaged by gamma-rays, particularly when the water content of the seeds is below 13 per cent. It may be worth noticing that the degree of radiosensitivity seemed to change most rapidly at water contents around 13 per cent.

In tetraploid rye, however, sensitivity started to decline when exposure exceeded approx. 42 K rads. Dry seeds were usually more sensitive to radiation than are seeds with higher moisture level. An opposite relationship was observed, however, for a moisture content of 28.0 per cent and a gamma-ray exposure between 28 K rads and 42 K rads in tetraploid rye. Similar relationship has been observed in other investigations (Gustafsson, 1947, Caldecott, 1955, a, b, c, Abrams and Frey, 1957, Ehrenberg, 1960). Regarding radiation sensitivity and plant growth, the results of this experiment seem to suggest the existence of very complicated interactions between the water content of seeds and radiation dosages regarding to radiation damage.

In both the varieties of rye germination was apparently unaffected by differences in gamma doses.

Doses suppressing 25 per cent of dry matter content in shoot for each level of water content and the 25% dose ratio between 4 x and 2 x has been presented in Table 2. The table shows for a water content of about 11 per cent, that chromosome doubling provided great protection against reduction of dry matter content in conjunction with irradiation with irradiation.

Table 2. The 25% dose ratio between 4 x and 2 x with respect to the dry matter content of the shoots in rye seedings after 10 days in germination.

25% dose ratio	H_2O level(%)	7.0	10.8	12.6	14.4	16.3	16.8	28.8
4x/2x		1.82	2.33	1.56	1.46	1.29	1.65	1.95

The second experiment intended to elucidate details in the effect of gamma irradiation on the growth of rye seedlings. In this second experiment, a number of dose levels, 3, 6, 8, 9, 12, 18, 24, 36 and K rads were investigated in addition to those studied the first experiment. Observations were made seven days after sowing and irradiation. The number of seeds in each treatment, environmental conditions and the method of sowing were equal to the arrangements made in the first experiment. The effects of gamma irradiation on shoot growth of both varieties of rye for seeds with different levels of moisture content are illustrated in the Fig. 3 and 4, while the effect on the dry matter content is presented in the Fig. 5 and 6.

Fig. 3 EFFECT OF Γ-RAYS AND MOISTURE CONTENT OF DIPLOID RYE SEEDS DURING IRRADIATION ON SUBSEQUENT SHOOT GROWTH

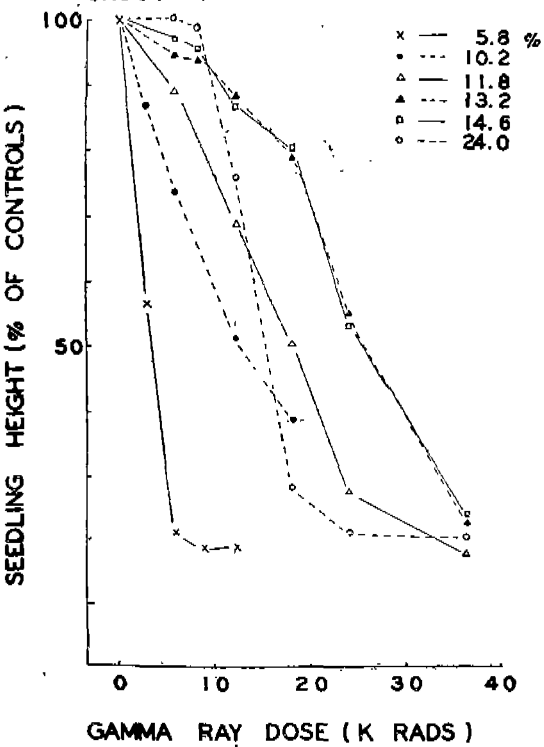
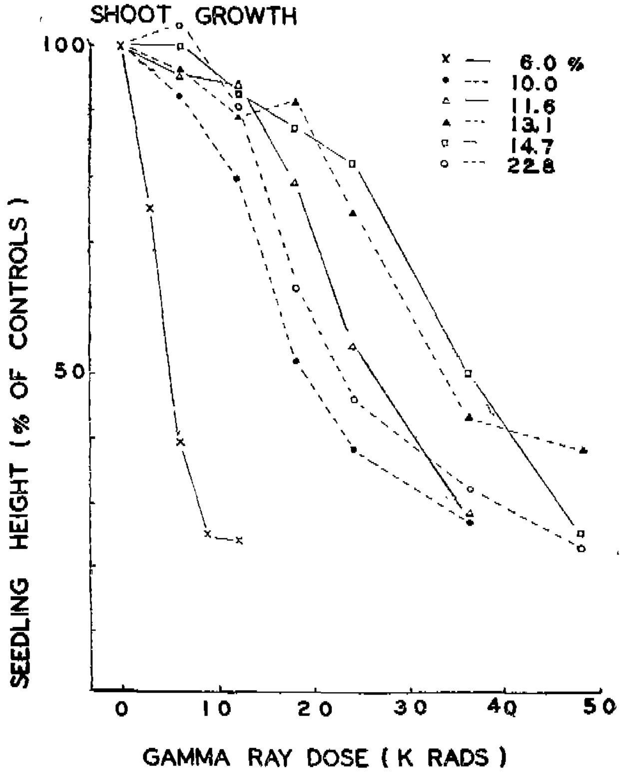


Fig. 4 EFFECT OF Γ-RAYS AND MOISTURE CONTENT OF TETRAPLOID RYE SEEDS DURING IRRADIATION ON SUBSEQUENT SHOOT GROWTH



When the mositure content of the seeds is very high, e.g., 23–24 per cent, the shoot growth was clearly stimulated after a dose of about 6 K rads. As observed in the first experiment, the sensitivity increased at moisture values exceeding 23–24 per cent.

Concerning the weight of oven—dried shoot(Figs. 5 and 6), the vertical dispersion is greater in diploid rye than that found in tetraploid rye for the same radiation dose at various levels of water content. The curves representing tetraploid rye thus tend to be close together save for the 6.0 per cent moisture level. The stimulative effect of gamma-rays on the average weight of oven-dried shoot has been shown

Fig. 5 AVERAGE DRY WEIGHT OF SHOOT OF SEEDLINGS

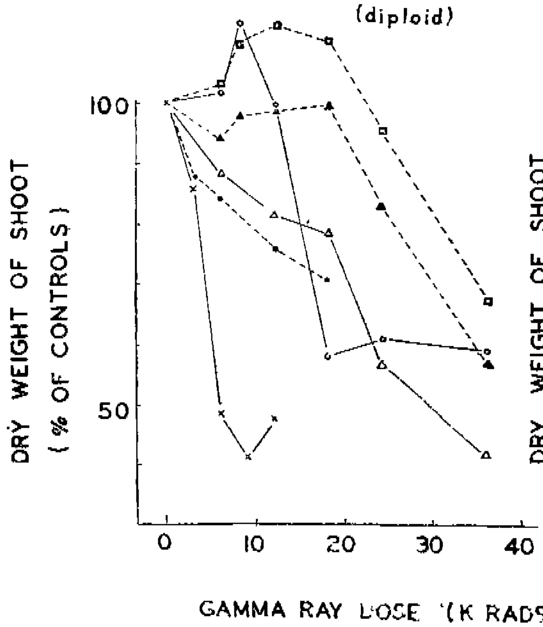
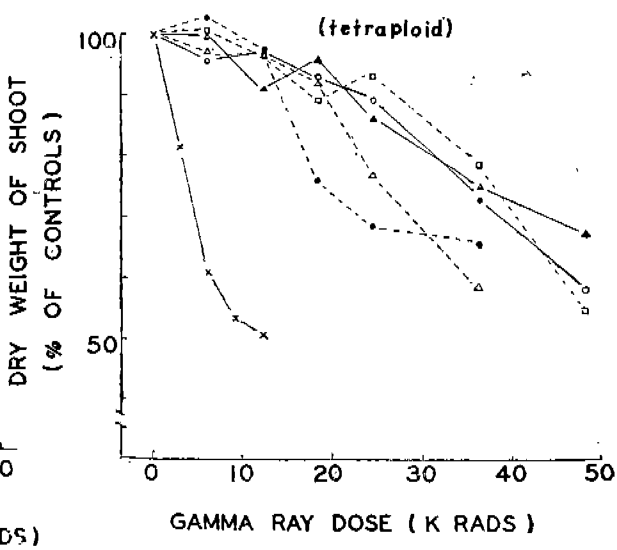


Fig. 6 AVERAGE DRY WEIGHT OF SHOOT OF SEEDLINGS



clearly. A peculiar feature in the correlation between the height growth and the dry matter growth of the shoot in the tetraploid rye variety may be of special interest. The height growth of seedlings developing from the seeds containing about 23 per cent of water, after a dose of 24 K rads ranks second from below, but the weight of oven-dried shoot, ranks second from above. Thus the seedling height growth and dry matter content of the shoot do not always run parallel, especially when the water content of the seeds is high. This feature is not so marked in the diploid rye.

It may also be worth considering whether differences in tolerance to radiation are caused by ploidy levels or by physiological, metabolic differences associated with the bigger size of seeds in the tetraploid variety, i. e., directly related to the amount of cytoplasmic content of the seeds. All the ratios of 50% dose for each level of water content were presented in Table 3 to investigate this point. Proportional correction of the 50% dose ratios is made for a water content of 24 per cent (by the method of interpolation).

Table 3. The 50% dose ratio between 4 x and 2 x, with respect to the shoot length in rye seedlings after seven days ln germination.

Hlevel % / 50% ratio	H_2O level(%)					
	6	10	12	13	15	24
4x/2x	1.34	1.50	1.45	1.30	1.39	1.48
Average of 4x/2x ratio: 1.41						

General Concerning the shoot growth developing from irradiated seeds, the N/X quotient amounted to about 20.0 in diploid rye, if a water content level of 10.0 per cent is equalled to air dry condition and if the 50% dose ratio is used for this quotient calculation. An N/X quotient 18.2 was obtained in tetraploid rye. These two values approximate rather two times of the N/X ratio of ten obtained by MacKey (1951) in barley for the cytological response to irradiation in the first mitotic cycle.

As tetraploid rye has four instead of two identical or similar genomes, the increased number of chromosomes per cell is apt to result in a higher absolute number of disturbances than that found in the related diploid rye, for the inductuion of a mutation by radiation is largely a chance event (Swaminathan and Natarajan, 1957, Nishiyama, et al., 1959, MacKey, 1960).

Polyploid rye showed great tolerance to radiation, neutron or X-rays in this experiments. This result agrees with other findings (Marshak and Bradley, 1944, Mikaelsen, 1958, Sparrow and Schairer, 19 59, MacKey, 1960, Breslavetz and Milesko, 1960). Thus it might be suggested as presented by MacKey (1960) that the gene duplication inherent in polyploidy acts as a buffer by increasing the radio-resistance. In these experiments, treated rye seeds germinated at a room temperature of about 22°C± 1. It is known that temperature is an important factor determining the level of radiation-induced damage in seeds. However, this statement seems to be slightly controversial. The survival of gamma-irradiated seeds of *Arabidopsis thaliana* was thus more depressed by a temperature of 27°C than by 20°C for both irradiated and non-irradiated material (Daly, 1960).

Whereas, germination at 27°C was conducive to lower damage in barley seeds irradiated by thermal energy, germination at a temperature of 20°C or lower seemed to induce greater damage (Konzak, et al., 1960). According to the Ehrenberg and Lundqvist experiment (1957) with X-rayed dormant barley seeds, differences between the germination temperatures 5°C and 20°C were observed with respect to frequency of mutations as well as sterility. The storage temperature as a post irradiation treatment is of great importance for the seedling length, the rate of chromosome aberrations, and fertility of spikes in Triticum (Matsumura, 1957). The influence of temperature at irradiation is similar to that of the germination temperature (Nybom, et al., 1953). Exact and constant temperature during germination must be kept to enable a correct evaluation of the results obtained.

CONCLUSIONS

The following conclusions may be drawn. Tetraploid rye was more tolerant to irradiation by fast neutrons than diploid rye. Root growth was more suppressed than was shoot growth at doses below 1120 rads in diploid rye and below 1400 rads in tetraploid rye. The germinative capacity was not influenced by this radiation. A sight stimulative effect on the dry weight of the shoot could be observed at very low doses of irradiation.

Diploid rye was more sensitive to gamma irradiation than was tetraploid rye. However, at rates exceeding 40 K rads in diploid rye and 60 K rads in tetraploid rye, the suppression of shoot growth was equal and independent of moisture content of the seeds used. The lower the moisture content of the seeds, the higher the radio sensitivity. Higher radiation sensitivity was again observed at 28 per cent moisture level.

The tetraploid/diploid ratio of doses decreasing the shoot growth by 50% was found to be about 1.6 for neutrons and the corresponding dose ratio for root growth inhibition was 2.5. This indicates that, since neutron damage is chiefly located to the nuclear material, cell division plays a relatively smaller role for the inhibition of the shoot growth. Accordingly a greater part of this growth is due to cell elongation. The tetraploid / diploid ratio of gamma doses producing 50% inhibition of the shoot growth was found to be about 1.4 independently of the water content at the irradiation.

This value is not very much lower than that found for the neutrons, indicating that nuclear damage is of nearly equal importance for the shoot growth inhibition produced by the densely ionizing neutrons. The 4×/2× ratio of neutron doses found for the seedling height of rye is appreciably lower than the corresponding value, 2.5 found by Ehrenberg and Nybom for barley. Further research is required to state whether cell division and cell elongation, respectively, are of different importance for the growth of the seedling of the two species. It must be remembered that the 4x/2x ratio is very sensitive to the *stage, at which the measurement occurs, and to the shape* of the seedling height-dose curve, which is not always the same for different types of radiation and for different species.

The germination percentage was rather unaffected by the dose of irradiation and the level of water content in seeds except when the seeds were equilibrated at very high moisture before irradiation. When water content was very high, exceeding 23 per cent, seedling height growth was stimulated by a dose of 6 K rads. Concerning the weight of oven-dried shoot, the influence of water content was more conspicuous in diploid rye than in tetraploid rye.

Concerning shoot growth, the effectiveness ratio of N/X equalled about 20.0 in diploid rye and about 18.2 in tetraploid rye, when a water content level of 10.0 per cent is equalled to air dry condition and when the 50% dose ratio is used for this quotient calculation.

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摘 要

二倍體 및 四倍體의 胡麥種子를 中性子 및 감마線에 照射해서 파종하고 發芽過程, 地上部와 地下部의 成長, 乾重量의 增加 等을 調査하였다. 특히 種子含水量이 放射線感受性에 대한 役割을 觀察하였다. 四倍體胡麥은 二倍體의 그것 보다 速中性子의 照射에 대해서 抵抗性이 더 强했다. 地下部는 二倍體胡麥에 있어서는 1120 rads, 四倍體에 있어서는 1400 rads 以下인데 地上部 보다 中性子照射에 대한 感受性이 높았다. 照射量이 낮을 때는 乾重量生長이 促進되는 效果가 있었다.

種子含水量이 낮을수록 放射線感受性이 增加했으나 含水量이 28%에 達했을 때 다시 回復되어서 높아졌다. 照射時 種子含水量이 23%를 초과하였을 때에는 6 K rads의 照射量에 있어서 地上部의 伸長生長이 促進되는 現象이 나타났고 地上部의 乾重量生長과 照射時의 種子含水量과의 關係는 四倍體胡麥 보다는 二倍體胡麥이 더 큰 影響을 받는 事實을 보았다.

地上部의 伸長量의 半減을 招來하는 照射量單位를 基準으로 할 때 N/X의 effectiveness ratio는 二倍體胡麥에 있어서는 약 20.0이였고 四倍體胡麥은 약 18.2였다.

LITERATURE CITED

1. Abrams, R. and J. Frey, 1957. The relationship between moisture content and X-ray sensitivity of oat seeds, Avena sativa. Proc. Iowa Acad. Science, 64 : 155-159.
2. Avanzi, S. 1960. Primi dati sulla rabioresistenza relativa dell ápice del germoglio e dell ápice della radice nel seme di orzo. Genetica Agraria, 13 : 113-122.
3. Breslavetz, L. P. and Z. F. Milesko, 1960. The defence of plants from ionizing radiation. Priroda, Leningrad, No. 5 : 50-53.
4. Caldecott, R. S. 1955 a. Effects of hydration on X-ray sensitvity in Hordeum. Rad. Res. 3 : 316-330.
5. Caldecott, R. S. 1955 b. Reduction in X-ray sensivity of seeds by hydration. Nature, 196 : 306.
6. Caldecott, R. S. 1955 c Effects of ionizing radiation on seeds of barley. Rad. Res. 2 : 339-350.
7. Daly, K. 1960. Effect of temperature on survival of gamma-irradiated *Arabidopsis* seed. Rad. Res. 12 : 430.
8. Ehrenberg, L. 1960. Induced mutation in plants: Mechanisms and Principles. Genetica Agraria, 12 : 364-389.
9. Ehrenberg, L., and D. von Wettstein, 1955. Radiation effects on the dry matter content in barley. Bot. Not. 108 : 216-230.
10. Ehrenberg, L. and N. Nybom, 1954. Ion density and biological effectiveness of radiations. Acta Agri. Scand. 4 : 396-418.
11. Ehrenberg, L. and U. Lundqvist, 1957. Post-irradiation effects in X-ray-induced mutation in barley seeds. Hereditas, 43 : 390-402.
12. Gray, L.H. 1946. Comparative studies of the biological effects of X-rays, neutrons and other ionizing radiations. British Medical Bulletin, 4 : 11-18.
13. Gustafsson, A. 1947. Mutations in agricultural plants. Hereditas, 33 : 1-100.
14. Kobayashi, Y. 1932. Wasserdampfspannung über wässerigen Lösungen von Kaliumhydroxid. J. Sci. Hiroshima Univ. A 2 : 269-274.
15. Konzak, C. F., H. J. Curtis, N. Delihas and R. A. Nilan, 1960. Modification of radiation induced damage in barley seeds by thermal energy. Canad. J. Cenet. Cyt. 2 : 129-141.
16. Mac Key, J. 1951. Neutron and X-ray experiments in barley. Hereditas, 37 : 421-464.
17. Mac Key, J. 1960 . Radiogenetics in "Triticum". Genetica Agraria, 12 : 201-230.
18. Marshak, A. and M. Bradley, 1944. X-ray inhibition of mitosis in relation to chromosome number. Proc. Nat. Acad. Sci., 30 : 231-237.
19. Matsumura, S. 1957. Effects of temperature and irradiation time upon mutations induced by radiations. The Annual Report of the National Institute of Genetics, No. 8 : 88-89.
20. Mikaelsen, K. 1958. The effect of polyploidy on the radio-sensitivity of rye seedlings exposed to neutron radiation. Rad. Res. 9 : 154.
21. Nishiyama, I., M. Okamoto, and T. Maruyama, 1959. Radiobiological studies on plants. Ⅲ. Sensitivity of the seeds of wheats and oats to gamma-rays from Co^{60}. Bull. of the Institute for Chemical Research, Kyoto Univ., 37 : 417-424.
22. Nybom, N., U. Lundqvist. A. Gustafsson, and L. Ehrenberg, 1953. Biological effects of X-irradiation at low temperatures. Hereditas, 39 : 445-459.
23. Sparrow, A. H. and L.A. Schairer, 1959. Some factors influencing radio-resistance and tumor induction in plants. Progress in Nuclear Energy Series. Vol. 2, Biological Sciences: 351-358.
24. Swaninathan, M.S. and A. T. Natarajan, 1957. Polyploidy and radiosensitivity. Nature, 179 : 479-480.