Note

Effects of 6 MeV Proton on Dry Seeds of *Chloris distichophylla*[†]

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Received September 2, 1980

Key Words: Proton, relative biological effectiveness, weeping chloris, dry seeds,
caffeine enhancement

1. Introduction

Recently, biological effects of high LET radiations and heavy ion radiations have been investigated from the standpoint of mutation induction or possible radiation hazard from these radiations during space flight^{1)~5)}. In the present experiment, the biological effects of 6 MeV protons and gamma-rays on plant material were investigated, because a few research work on the effect of proton to plants has been done.

1.1 Sample and sample preparation

Dry seeds of weeping chloris, *Chloris distichophylla*[®], strain 52-00-01 were used as a sample, because the size of this seed was small (average 0.8×1.5 mm) and genetically homogeneous. They were kindly supplied by the National Seed Storage Laboratory for Genetic Resources of Japan. Forty seeds were placed on the stainless steel mesh as one group in each dose and sealed with formbal (mixture of dichloroethane and polyvinylformal; ca. 5~100 nm (50~1000 Å) thick) on the mesh.

1.2 Irradiation

[†] *Chloris distichophylla* の乾燥種子に対する 6 MeV プロトンの影響。田野茂光, 江口星雄*, 山根功*, 山下博*, 山口彦之: 東京大学農学部, *東京大学原子力研究総合センター, 113 東京都文京区弥生 2-11-16, 東京大学原子力研究総合センター内

Stainless steel mesh with sample was fixed on the holder and placed vertically along the sector at the specified degree in the irradiation chamber, where the samples were exposed to the radiation produced by Rutherford scattering. Scattered protons of 6 MeV were generated with a Tandem type van de Graaf accelerator at the Research Center for Nuclear Science and Technology, The University of Tokyo. Dose range of proton irradiation was 0 to 1 kGy (100 krad). Dosimetry of proton was performed with a silicon solid-state detector placed in the irradiation chamber.

Gamma irradiation was performed with ¹³⁷Cs gamma-rays ranging from 0 to 15.48 C/kg (0 to 60 kR) and the dose intensity was 129 mC/kg (500 R) per min.

1.3 Observation of irradiated samples

Each irradiated and unirradiated sample was germinated on the filter paper in petri dishes at 25°C immediately after irradiation. A part of the proton irradiated and unirradiated seeds was treated with 5 mM caffeine solution for 5 hours prior to germination to detect the inhibition of recovery. Percentages of germination and plant height after 21 days were measured and used as an index for comparison of the biological effects of proton and gamma-rays.

2. Results

2.1 Proton irradiation

Figure 1 shows the dose response curve of the germination to proton irradiation. As seen from this Fig., no clear decrease in germination was observed up to 50 Gy (5 krad); however, sharp decrease in germination was clear when the dose exceeded 50 Gy (5 krad). Treatment with 5 mM caffeine enhanced the decrease in germination percentages even in the range of low doses less than 50 Gy (5 krad).

Fig. 1 Effect of proton irradiation on germination. Closed circle: without caffeine, open circle: 5 mM caffeine treated. (1 rad=10 mGy)

Height of survived seedlings measured at the 21 days after germination showed the same response to the germination against dose in the case of proton irradiation; however, no clear linear inhibition with caffeine was observed in this index (Fig. 2).

Fig. 2 Effect of proton irradiation on plant height. Symbols are the same in Fig. 1. (1 rad=10 mGy)

2.2 Gamma-ray irradiation

Figure 3 shows the dose response curves of germination and plant height in the case of

gamma-irradiation. Dose response curves of germination percentage and plant height was clearly linear in the entire dose range.

Fig. 3 Effect of gamma-rays on germination and plant height. Open circle: germination, closed circle: plant height. (1 kR=258 mC/kg)

3. Discussion

No clear decrease in germinability was observed between 0 and 50 Gy (0 and 5 krad) of proton irradiation. However, marked decrease was observed when the dose exceeded 100 Gy (10 krad). Germinability was reduced to about 30% of the unirradiated samples after 1 kGy (100 krad) irradiation. Bettega et al.²⁾ reported the effect of 12 MeV protons on the formation of micronucleus and multinucleate cells in cultured human cells. The frequency of both effects was increasing function to the dose. They showed the dose response curve was estimated as an exponential function. In the present experiment, the relationship between dose and biological effects was seemed to be exponential.

Treatment with 5 mM caffeine to the irradiated seeds enhanced the damage induced by proton irradiation, even at lower dose range. This suggested that some amount of damages caused by proton irradiation was repairable.⁷⁾

Effect of proton irradiation on plant height measured 21 days after irradiation showed similar response curve to germinability. However, effects on growth were slightly higher than on germinability in the low dose range. Effects of proton

observed at the highest dose on germinability and plant height were almost the same, i.e., about 70% reduction of the control values.

Slater and Tobias⁴⁾, and Curtis and Smith⁵⁾ independently reported some developmental abnormalities in maize grown from the seeds which were experienced space flight. This effect was attributed to the passage of heavy ion. A significant effect on growth of plants by heavy ion irradiation was reported on maize. Yang and Tobias¹⁾ reported that the seeds irradiated with 10 Gy (1 krad) of oxygen particles grew into plants about 1/4 the size of the controls.

Seeds kept in vacuo for about 6 hours for proton irradiation did not show any damage on germinability and plant height compared to that was kept under natural atmospheric condition.

In the case of gamma irradiation, clear linear decrease curves were obtained between 1.29 and 15.48 C/kg (5 kR and 60 kR) on germinability and plant height.

Relative biological effectiveness of proton to gamma-rays were estimated using germinability and plant height. The estimated RBE values were 1.12 obtained from 30% reduction of germination and 1.44 from 30% reduction of plant height.

Baily and Steigerwalt⁸⁾ stated that many investigators reporting measurement of RBE using X-rays, gamma-rays and electrons, there was a fair degree of consistency for the values. However, in the case of protons, these consistency did not exist even the same investigators using identical beams. They suggested the

difference in RBE value in the case of proton may be due to differences in recovery factors, site size or in certain instances the existence of an energy threshold for observable damage to take place. They also showed that expected RBE calculated from the value of the ratio of average to most probable energy losses varies depending on the energy of electrons taken as the reference.

In the present experiment, RBE values were different with different estimation indices; however, these values were fairly close to the reported values in mammalian cells.

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