Effect of Ar-ion beam irradiation on imbibed seed of rice

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Linear energy transfer (LET) is an important factor for mutation induction by heavy-ion beam irradiation. The following ions are applicable for irradiation of biological samples at the RIBF: C, N, Ne, Ar, and Fe. The LET values of these ions calculated at the surface of the sample are 22.5, 30.0, 61.5, 280.0, and 624.0 keV/μm, respectively. It is possible to control the LET value by selecting a particular ion or using a range shifter of automatic irradiation system[1]. It has been reported that high-LET irradiation causes large deletion or chromosome aberration in Arabidopsis[2-4]. Furthermore, our previous study revealed that Ar-ion irradiation causes larger deletion than C- or Ne-ion irradiation in rice[5]. In this study, we estimated the effect of high-LET irradiation on the mutation efficiency in rice using an Ar-ion beam.

Imbibed seeds of rice (*Oryza sativa* L. cv. Nipponbare) were exposed to Ar-ions accelerated to 95 MeV/u at the RIBF. The dose range of the Ar-ion beam (LET 290 keV/μm) was 5 to 20 Gy. The irradiated seeds were sown in soil in pots and grown in a greenhouse. The survival rates were investigated for two-week old seedlings. We estimated the optimum dose for mutation induction from the results of the survival rate. The seed fertilities and mutation frequency were observed in two experiments after irradiation with pre-determined doses. The first experiment was a comparison between doses of 2,5 Gy and 5 Gy. The second one was a comparison between doses of 5 Gy and 7.5 Gy. The number of irradiated seeds for each dose was 750. The seed fertility was evaluated using the percentage of fertile seeds per panicle in the first experiment, and using the number of fertile seeds in one panicle in the second experiment. The mutation frequency was calculated using the percentage of M_1 lines, which showed chlorophyll deficient mutants (CDM) in M_2 progenies.

The survival rate of M_1 seedlings significantly decreased for doses over 10 Gy (Fig. 1). The growth inhibition was more severe as the dose increased (Fig. 2). These results indicate a higher inhibition effect of Ar-ions on growth and survival compared to C-ions[6]. The effect of dose on fertility in M_1 plants is shown in Fig. 3. The ratio of M_1 plants with low fertility increased as the dose increased. Nearly 40% of the M_1 plants had less than fifty seeds per panicle at a dose of 7.5 Gy.

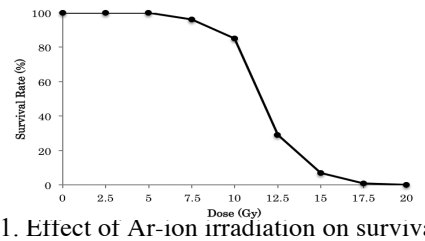

Fig. 1. Effect of Ar-ion irradiation on survival rate.

The mutation rates obtained with Ar-ion irradiation were 6.1%-8.7% (Table 1). These results are almost the same as those of C-ion irradiation (LET:50 keV/μm) with a dose of 15 Gy[6]. Mutation frequency is the most important factor for determining the effective condition in mutagenesis. However, seed fertility is also an important factor because sterility is an undesirable trait for breeding. Furthermore, a low fertility increases the risk of outcross in the field. Considering the results of both factors, irradiations with doses of 2.5 Gy and 5.0 Gy is adequate for mutation breeding using Ar-ions in rice.

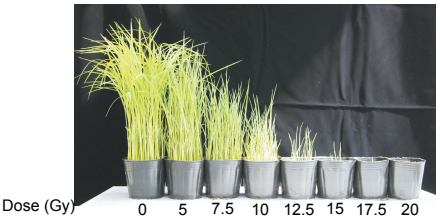

Fig. 2 Growth condition of M_1 seedlings after 2 week.

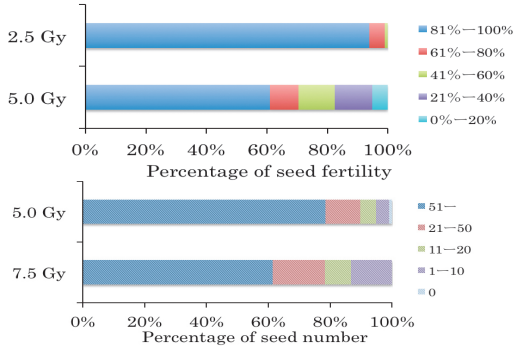

Fig. 3 Effect of dose on seed fertility in M_1 plants. The average seed fertility and fertile seed number in control Nipponbare were 92% and 108.6, respectively.

Table 1 Effect of Ar-ion irradiation on survival rate and mutation frequency

Dose (Gy)	Fertile M_1 line	Survival Rate (%)	Total CDM	Frequency of CDM
2.5	686	91.5	42	6.1
5	684	91.2	42	6.1
5	690	92	49	7.1
7.5	623	83.1	54	8.7

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