Comparison of LET effect of heavy-ion beam irradiation in rice

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Linear energy transfer (LET) is a contributing factor in heavy-ion mutagenesis. Our previous study revealed a LET-dependent effect in *Arabidopsis thaliana*. A LET of 30 keV/μm is the most effective for inducing mutation.[1)] Although a high LET of heavier ions such as Ar and Fe resulted in a lower mutation frequency, they can easily cause larger deletions and complex mutations.[2–5)] In 2015, a new high-energy beam line called the WACAME line was constructed, which enables the irradiation of heavier ions with a longer range.[6)] The choice of LET values for biological samples was extended by using this beam line. Knowledge about the LET effect in model plants can be applied for other plants and is useful for efficient mutagenesis. In this study, we examined the effect of a higher LET of heavy-ion beam irradiation in rice as another model plant.

Dry seeds of rice (*Oryza sativa* L. cv. Nipponbare) with a water content of 13% were used for the experiment. The seeds were placed into a plastic bag without overlapping and vacuum-packed for irradiation treatment. The seeds were irradiated with Ar (184 keV/μm, 289 keV/μm) and Fe (650 keV/μm). Ar (184 keV/μm) is a high-energy beam produced using the WACAME line. The doses of Ar ions and Fe ion were 10 to 50 Gy, 7.5 to 40 Gy, and 10 to 50 Gy, respectively. Survival rates were estimated by counting plants surviving four weeks after sowing. Figure 1 shows the survival curve after irradiation. A decrease of survival rate was observed for a dose greater than 20 Gy with Ar (289 keV/μm) irradiation. On the other hand, a decrease of survival rate was observed for a dose greater than 30 Gy with Ar (184 keV/μm) and Fe (650 keV/μm) irradiation. M_1 plants from the irradiation with a high survival rate were grown in a paddy field. M_2 seeds were obtained individually from each M_1 plant. The fertility was evaluated using the number of fertile spikelets in the main panicle of the M_1 plant. Figure 2 shows the percentage of fertility in M_1 plants. The mean value of the number of fertile spikelets per panicle in control Nipponbare was 105.7 $\pm$ 16.24. The percentage of low-fertility plants increased with the increase of dose at any LET value.

Chlorophyll-deficient mutants (CDM) were observed in two-weeks-old M_2 seedlings grown in a greenhouse. Mutation rates were calculated based on the numbers of M_1 lines, which showed CDM in M_2 generation. The highest frequency of CDM was observed at 20–30 Gy of Ar (184 keV/μm) irradiation (Table 1). Furthermore, there was no severe decrease of fertility at a dose of 20 Gy. This result suggests the possibility of high efficiency of the Ar-ion beam produced using the WACAME line. We will conduct the genome sequencing of isolated mutants to characterize the mutations irradiated at 184, 289, and 650 keV/μm and compare the mutagenic effects of higher-LET irradiation in rice.

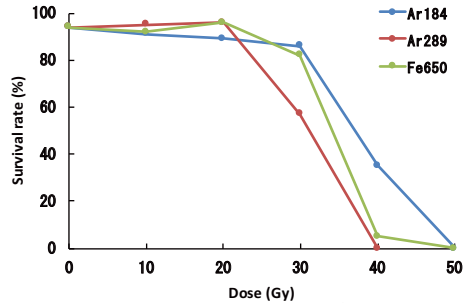

Fig. 1. Effect of LET on survival rate in rice.

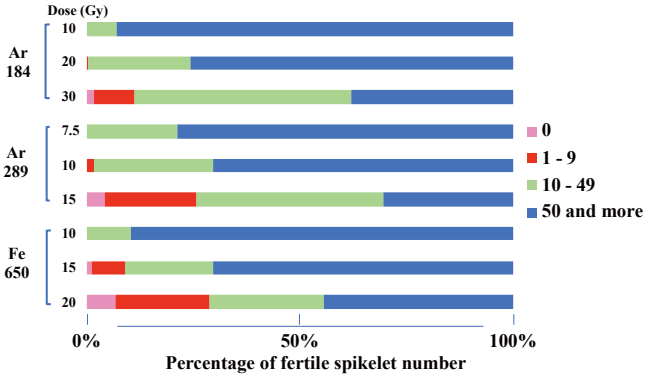

Fig. 2. Comparison of the number of fertile spikelets per panicle.

Table 1. Effect of High-LET ion-beam irradiation on mutation induction.

Ion	LET (keV/μm)	Dose (Gy)	Mutation rate (%)	Number of CDM: Albino	Pale green	Stripe	Yellow	Others
Ar	184	10	10.28	16	7	1	0	5
		20	11.63	12	10	5	0	3
		30	11.98	16	4	4	0	5
Ar	289	7.5	6.28	8	5	1	0	1
		10	8.37	9	5	2	0	4
		15	9.28	11	7	2	0	2
Fe	650	10	4.18	3	3	2	0	2
		15	4.66	6	1	0	1	3
		20	10.17	11	8	3	1	1

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