Optimization of mutation induction by heavy-ion beam irradiation in rice with seed water content change

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Radio sensitivity to X-rays and γ-rays are minimal at intermediate water contents in seeds of rice,[1), 2)] wheat and barley.[3)] It increases rapidly above and below this range. In contrast, the seed water content has little or no effect on the sensitivity to fission neutron irradiation.[2)] Our previous study revealed that the radio sensitivity to C-ion beam was affected by the water content of seeds as in the cases of X-rays and γ-rays in rice.[4)] In this report, we investigated the effect of seed water content on mutation rate by C-ion irradiation and estimated the efficient irradiation condition for optimized mutagenesis in rice.

The dry seeds of rice (*Oryza sativa* L. cv. Nipponbare) were equilibrated to 9% and 13% water contents through storage at constant humidity. Water-content-adjusted seeds were vacuum-packed in plastic bags for irradiation treatment (Fig. 1). The dose ranges of the C-ion beam were determined according to our previous study[4)] as 12.5 to 50 Gy and 100 to 200 Gy for 9% and 13% water content, respectively. The liner energy transfer (LET) values were 22.5, 30, and 50 keV/μm at each irradiation dose. M_1 plants were grown in a paddy field and M_2 seeds were obtained separately from each M_1 plant. The number of surviving M_1 plants after each treatment was counted to estimate survival rates. The spikelet fertility was evaluated using the number of fertile spikelets in the main panicle of the M_1 plant. Table 1 lists the percentage of low-fertility M_1 plants that have less than fifty fertile spikelets in a panicle. The mean value of the number of fertile spikelets per panicle in control Nipponbare was 106.2 ± 12.2. 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.

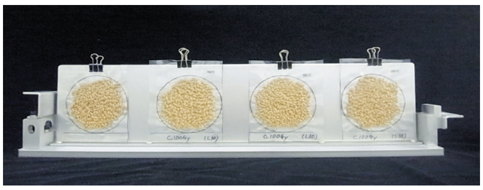

Fig.1 Rice seeds set into a cassette of automatic sample changer in our irradiation system.[5)] Each bag contains three hundreds seeds of Nipponbare.

The results of survival rate, spikelet fertility, and mutation rate are listed in Table 1. In the irradiation on seeds with 9% water content, a higher frequency of CDM was obtained by high dose irradiation, which resulted in a low survival rate. In contrast, the highest frequency of CDM was obtained by the irradiation on seeds with 13% water content without the extreme reduction of both survival rate and spikelet fertility. There was no difference between seeds with 9 % and 13% water content in the responses to LET. Irradiations with 50 keV/μm were more effective than the same doses of irradiation with 22.5 or 30 keV/μm on the survival rate and spikelet fertility. As a result, we determined that the optimum condition of C-ion irradiation for mutation induction was 175 Gy (30 keV/μm) on seeds with 13% water content. In general, rice seeds are recommended to be stored under cold and dry conditions to maintain its activity. Therefore, the water content after long-term storage tends to be less than 9%. It is indispensable to measure the water content of seed before irradiation to set an appropriate condition in response to its water content. To induce mutation with high probability, we adjust the water content to 13% by keeping the seeds in a thermo-hygrostat for a week under a temperature of 22°C and humidity of 70%.

Table 1. Effect of C-ion beam irradiation on seeds with 9% and 13% water content.

Water content	LET (keV/μm)	Dose (Gy)	No. of survived M_1 plant	Survival rate (%)	Rate of low-fertility M_1 plant (%)	CDM rate (%)
9%	22.5	25	499	83.2	4.0	8.0
		50	428	71.3	11.7	8.9
	30	25	505	84.2	3.1	6.9
		50	388	64.7	18.1	9.8
	50	12.5	214	71.3	7.0	5.6
		25	151	50.3	17.2	9.9
		50	161	53.7	11.8	10.6
13%	22.5	125	99	99.0	7.2	9.09
		150	346	86.5	19.9	11.10
		175	348	87.0	24.7	14.10
		200	96	96.0	13.5	9.38
	30	125	97	97.0	3.1	8.25
		150	339	84.8	8.3	10.60
		175	325	81.2	19.7	15.10
		200	97	97.0	35.0	8.25
	50	100	94	94.0	10.6	5.32
		125	307	76.8	37.1	11.70
		150	175	43.6	48.6	11.40
		175	53	53.0	37.7	7.55

References
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