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Effect of heavy-ion beam irradiation on mutation induction in *Arabidopsis thaliana*

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Since high linear energy transfer (LET) heavy-ion beams cause clustered damages including DNA double-strand breaks,[1] they seem to be more effective for inducing mutations than low LET radiation such as X-rays, γ-rays and electrons. In basic plant sciences, mutants defective in a biological process provide useful tools for genetic analysis. Arabidopsis (*Arabidopsis thaliana*) is one of the model plants and often used in such genetic analyses. To achieve effective mutagenesis of arabidopsis, we evaluated the conditions of heavy-ion beam irradiation on mutation induction.

Dry seeds of arabidopsis ecotype Columbia (about 250 μm in diameter) were packed with Hybri-Bag Hard (95 μm thickness, Cosmo Bio Co., Ltd.) to provide a monolayer of the seeds for homogenous irradiation. They were irradiated with ^{12}C, ^{20}Ne, ^{40}Ar and ^{56}Fe ions with a dose range of 5 to 400 Gy. These ions were accelerated up to 135, 135, 95 and 90 MeV/nucleon, and their LETs were 23, 61, 280 and 630 keV/μm, respectively. Furthermore, to determine the effects of the LETs of heavy ions on mutagenesis, the LET of C ion beams was increased to 63 keV/μm after passing through a set of absorbers in the range shifter. All LETs were calculated at the seed surface. The irradiated M_1 seeds were incubated on 1/2 MS agar medium at 4°C in darkness for 3 days for vernalization and then at 22°C under continuous illumination. The germination rate (number of germinated seeds per total number of incubated M_1 seeds) was determined 2 to 3 weeks after the initiation of incubation. True leaf-developed seedlings were transplanted to plastic trays (13 × 9 cm) containing soil. Eleven seedlings were planted in each tray and cultured at 22°C under continuous illumination in the greenhouse. The flowering rate (number of flowering plants per total number of incubated M_1 seeds) was determined one month after the transfer. M_2 seeds were harvested from self-pollinated M_1 plants in each tray. M_2 seeds were similarly collected and incubated under the same condition as that for germinating M_1 seeds. The frequencies of albino plants (number of albino plants per total number of germinated M_2 seedlings) were measured after most seedlings expanded cotyledons.

In the control seeds without irradiation, the germination rate was 97.1% and all seedlings flowered until one month after transfer to soil. The effects of heavy-ion beam irradiation on mutagenesis in arabidopsis are shown in Table 1. Germination of M_1 plants was not affected by irradiation with any ions at the doses tested. However, the decrease in the flowering rate of M_1 plants was observed in accordance with the increase in doses. For most M_1 plants after high-dose irradiation, growth-decreased plants produced a few seeds. Among the irradiation conditions using the different energies of C ions, LET of 63 keV/μm was more effective for inducing albino plants. Similar frequencies of albino plants were observed for both irradiation treatments by C ions with LET of 63 keV/μm and by Ne ions with LET of 61 keV/μm. Also, the same doses were required for higher frequencies. It has been reported that the lethality rate after ion beam irradiation was affected by LET in arabidopsis: Ne or Ar ions with LET higher than 350 keV/μm were more effective than 113 keV/μm C ions.[2] The LET of ion beams seems to be an important factor for the efficient mutagenesis of plants. More detailed studies using several ions at different LETs are in progress.

In the present study, not only albino plants but also several morphologically abnormal plants, such those with variegation, pale green color, longer hypocotyl and higher anthocyanin accumulation, were isolated in the M_2 generation derived from seeds irradiated by all ion beams tested. Molecular analyses of mutated genes to clarify the effect of ions and their LETs are also in progress using these mutants.

Table 1. Effect of heavy-ion beams on mutation induction in *Arabidopsis*.

Ion	LET (keV/μm)	Dose (Gy)	No. of M_1 seeds§1	Germination rate of M_1 plants (%)	Flowering rate of M_1 plants (%)	% of albino M_2 plants
Control	–	–	680	97.1±2.5	97.1±2.5	0.07±0.07
C	23	50	371	93.5±4.0	93.5±4.0	0.16±0.1
		100	256	86.0±2.5	83.1±0.8	0.48±0.4
		150	210	89.3±3.9	87.3±4.3	0.34±0.1
		200	279	94.2±1.0	81.2±0.7	0.53±0.1
		400	255	91.1±4.2	34.2±11.6	nd§2
C	63	50	439	95.8±0.5	94.8±0.6	0.80±0.1
		100	455	98.1±0.6	93.1±1.8	1.35±0.4
		150	446	92.3±3.7	91.4±3.2	1.92±0.4
		200	413	98.2±0.5	19.2±17.1	nd
		400	358	96.0±1.6	0.8±0.1	nd
Ne	61	50	515	97.6±0.4	94.9±1.9	0.30±0.2
		100	412	92.9±3.9	91.8±3.5	1.02±0.4
		150	390	91.8±5.4	90.3±4.4	1.67±0.7
		200	393	88.5±7.9	83.2±8.9	0.39±0.1
		400	301	87.2±6.3	3.0±1.9	nd
Ar	280	5	580	97.9±1.1	89.5±2.1	0.20±0.1
		10	567	94.2±5.3	88.2±4.8	0.32±0.1
		20	479	94.6±4.3	91.4±4.3	0.26±0.1
		50	499	97.6±2.1	92.2±2.0	1.37±0.3
		100	445	93.9±4.0	1.2±1.2	nd
		150	452	92.6±5.9	0	nd
		200	341	95.5±3.7	0	nd
Fe	640	5	410	97.0±0.3	96.6±0.2	0.10±0.1
		10	408	93.8±1.9	93.5±2.0	0.35±0.3
		20	341	92.9±4.2	92.9±4.2	0.17±0.1
		50	324	95.6±3.0	94.4±3.1	0.42±0.2
		100	226	94.1±1.8	74.9±4.7	1.09±1.0
		150	196	91.4±3.4	5.9±5.9	nd
		200	210	86.9±6.4	0	nd

§1 Total number of irradiated M_1 seeds from three experiments.
§2 Data were not determined.

References

1) D. T. Goodhead: Int. J. Radiat. Biol. **65**, 7 (1994).
2) N. Shikazono et al.: Radiat. Environ. Biophys. **41**, 159 (2002).