LET-dependent effect on mutation induction DNA repair-deficient background in *Arabidopsis thaliana*

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A previous study on the effect of LET on inducing mutation revealed that the most effective value of LET (LETmax) on dried seeds of *Arabidopsis thaliana* was 30.0 keVμm^{-1}, within a range 22.5 to 640 keVμm^{-1}.[1)] LET is therefore an important factor in mutation induction. In the mutagenesis process, the DNA double-strand break (DSB) repair system is concerned with the production of mutations. There are two major DSB repair pathways: non-homologous end joining (NHEJ) and homologous recombination repair (HRR) function in eukaryotic cells.[2)] NHEJ and HRR are independent pathways. HRR is a relatively error-free pathway because it utilizes the homologous region of a sister chromatid to repair the damaged strand, whereas the NHEJ pathway is relatively error-prone.

To determine whether DSB repair pathways are involved in the LET-dependent effect on mutation induction, we intended to investigate the effect of LET in the DSB repair-deficient background. We planned to measure the mutation rates after heavy-ion beam irradiation with LET values 22.5 and 30 keVμm^{-1} in the three mutant lines: 1) the HRR pathway-deficient mutant line, 2) the NHEJ pathway-deficient mutant line, and 3) both HRR and NHEJ pathway-deficient mutant line. Here, we report the mutation rates of the *Rad54*-deficient mutant as the HRR pathway-deficient mutant line.

Seeds of the *APG3*$^{(+/-)}$ mutant (CS16118) and the *AtRad54*$^{(-/-)}$ mutant (SALK_038057) were obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University) and the European Arabidopsis Stock Centre (NASC, the University of Nottingham), respectively. The *APG3*$^{(+/-)}$ mutant carries BASTA-resistance at the *APG3*-disrupted allele, and a uniformly heterozygous population can be selected as photosynthetic and BASTA-resistant seedlings.[3)] The uniformly heterozygous population facilitates investigation of the mutation frequency in the irradiated (M_1) generation by calculating the proportion of the number of plants with white sectors on true leaves to that of total plants with true leaves (Fig. 1). The *AtRad54*$^{(-/-)}$ mutant carries a kanamycin resistance. The *APG3*$^{(+/-)}$ plants were crossed with the *AtRad54*$^{(-/-)}$ plants. The F_1 seeds were germinated in the presence of BASTA (2 μg/mL) and kanamycin (50 μg/mL), and the germinated plants were replanted to pots. F_2 seeds were collected from the self-pollinated F_1 plants. In the F_2 generation, the photosynthetic and both BASTA- and kanamycin-resistant plants were screened. The second screening of the *APG3*$^{(+/-)}$/*AtRad54*$^{(-/-)}$ plants were conducted by PCR. A sufficient number of seeds were collected from progenies of the identified *APG3*$^{(+/-)}$/*AtRad54*$^{(-/-)}$ plants.

Heavy-ion beam irradiation was conducted as previously described[3)] with some modifications. The seeds of the *APG3*$^{(+/-)}$/*AtRad54*$^{(-/-)}$ and *APG3*$^{(+/-)}$/*AtRad54*$^{(+/+)}$ plants were irradiated with $^{12}C^{6+}$ ions with LETs of 22.5 or 30.0 keVμm^{-1} at a dose of 300 Gy.

The mutation frequencies were obtained as previously described.[3)] The mutation frequencies of *AtRad54*$^{(+/+)}$ plants were 3.0 and 6.6% when the LET values were 22.5 and 30.0 keVμm^{-1}, respectively, and they are significantly different (Table 1; $p<0.05$ with chi-square test). The mutation frequency of 22.5 keVμm^{-1}-irradiated *AtRad54*$^{(-/-)}$ plants was 5.6%, which was at the same level as that of 30.0-keVμm^{-1} irradiated control ($p\geq0.05$). It is assumed that because the HRR pathway is disabled, the error-prone NHEJ pathway mainly functioned to repair DSB. The mutation frequency of 30.0-keVμm^{-1} irradiated *AtRad54*$^{(-/-)}$ plants, however, was still 6.8%, which was at the same level as that of the control ($p\geq0.05$). This result proposed a hypothesis: in the case of the 30.0-keVμm^{-1} irradiation, in contrast to the 22.5-keVμm^{-1} irradiation, DSBs occur beyond the capacity of the HRR pathway functions and are repaired mainly by the NHEJ pathway, leading to a high mutation frequency. Further analysis on other DNA repair gene-deficient mutants is in progress.

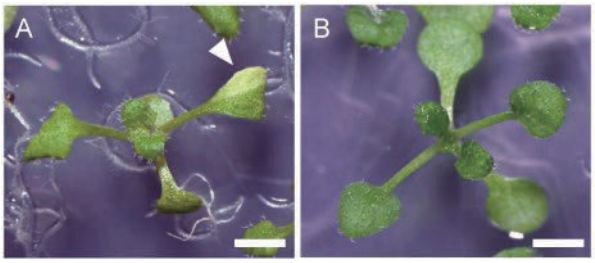

Fig. 1. Sector mutation caused by heavy-ion beam irradiation on the *APG3*$^{(+/-)}$ mutant. (A) A plant showing sector mutation. The leaf exhibiting mutation is indicated by the white arrowhead. (B) A plant not showing abnormal phenotype. Bars = 2 mm.

Table 1. LET-dependent effect on inducing mutation.

LET (keVμm^{-1})	Mutation frequency (%)	
	AtRad54$^{(-/-)}$	*AtRad54*$^{(+/+)}$
22.5	5.6 (916)	3.0* (2,241)
30.0	6.8 (857)	6.6* (2,118)

Numbers in parentheses indicate numbers of samples. *Kazama et al. (2012).

References

1) Y. Kazama et al.: Plant Biotechnol. 25: 113-117 (2008).
2) L. H. Thompson: Mutat. Res. 751: 158-246 (2012).
3) Y. Kazama et al.: Plant Biotechnol. 29: 441-445 (2012).

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