Contribution of multiple DNA repair pathways in *Arabidopsis thaliana* dry seeds after heavy-ion-beam irradiation

K. Ishii,[*1] S. Ohbu,[*1] M. Yamada,[*1] and T. Abe[*1]

Heavy-ion-beam irradiation induces DNA double-strand break (DSB) which is critical damage to cellular proliferation. DSB is repaired by two main pathways: homologous recombination (HR) repair, which is homology-dependent and error-free; canonical non-homologous end-joining (C-NHEJ), which is Ku-dependent and potentially error-prone and by a recently identified error-prone pathway, alternative non-homologous end-joining (A-NHEJ). Which pathway is chosen is dependent on the phase of cell cycle and the initiation of DNA end resection.[1] HR is preferred during the S/G2 phase in the cell cycle because sister chromatids can be used as homologous templates. C-NHEJ functions through the cell cycle and especially in G1 and G2 phase. A-NHEJ functions when the key factors of C-NHEJ are absent, essentially in the S phase. Because most embryo cells in dried seeds are in the G1 phase,[2] it is possible that C-NHEJ will mainly function after heavy-ion-beam irradiation. In this study, we irradiated heavy-ion-beams on three different lines: a wild type, a C-NHEJ-deficient line, and a HR-deficient line, and we measured mutation rates to reveal how each DSB-repair pathway functions.

We obtained *Arabidopsis thaliana* seeds of SALK_123114 line with a T-DNA insertion in the *Ku*70 gene (a *Ku*70(−/−) mutant) and SALK_038057 line with that in the *Rad*54 gene (a *Rad*54(−/−) mutant) from Nottingham Arabidopsis Stock Centre (NASC) and used them as the C-NHEJ-deficient and HR-deficient lines, respectively. Dry seeds of Col-0 line (wild type), the *Ku*70(−/−) mutant, and the *Rad*54(−/−) mutant were irradiated with $^{12}C^{6+}$ (135 MeV/nucleon) ions at doses ranging from 0 to 600 Gy. The LET of C-ion beams were controlled to 30 keV/μm. After the irradiation, flowering rate (number of flowering plants per total number of incubated seeds) were measured as survival rate, as previously described.[3] We also obtained seeds of CS16118 line with a T-DNA insertion in the *APG*3 gene (an *APG*3(+/−) mutant) from NASC. The *APG*3(+/−) mutant was crossed with the *Ku*70(−/−) and *Rad*54(−/−) mutants to produce *Ku*70(−/−)*APG*3(+/−) and *Rad*54(−/−)*APG*3(+/−) mutants in the F_2 generations, respectively. Dry seeds of the *APG*3(+/−) line (regarded as a wild type), *Ku*70(−/−)*APG*3(+/−) line, and *Rad*54(−/−)*APG*3(+/−) line were irradiated with $^{12}C^{6+}$ (135 MeV/nucleon) ions at 60 Gy. The LET of C-ion beams was controlled to 30 keV/μm. After the irradiation, appearance ratio was measured as mutation rate, as previously described.[4]

The flowering rates of un-irradiated control of the wild type, C-NHEJ-deficient line, and HR-deficient line were not lower than 0.99. In the wild type, the flowering rate was 0.80 even after 600 Gy-irradiation, while in the *Rad*54(−/−) mutant, the rate was reduced to 0.30. In the *Ku*70(−/−) mutant, there was no individual flowered only after 120 Gy-irradiation. It is suggested that both C-NHEJ and HR pathways are involved in DNA repair after heavy-ion-beam irradiation and the C-NHEJ pathway has a greater contribution.

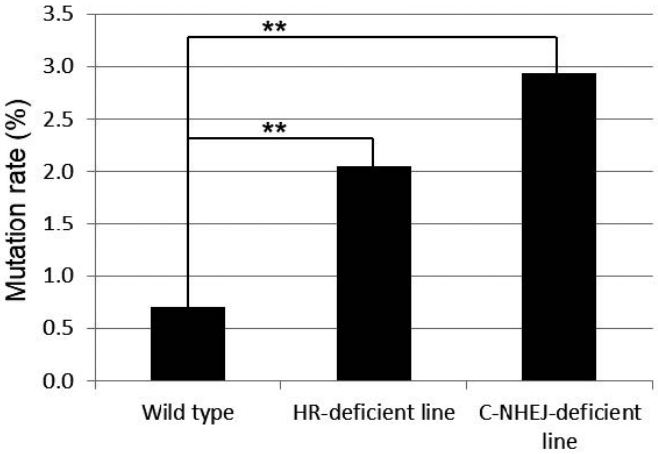

Fig. 1. Mutation rate after C-ion-beam irradiation at 60 Gy. **: $p < 0.01$.

While the mutation rate of the wild type was 0.7%, those of the HR-deficient and C-NHEJ-deficient lines were significantly ($p < 0.01$) higher: 2.0% and 2.9%, respectively. It is possible that in the HR-deficient lines, DSBs usually repaired by the HR pathway are repaired by the C-NHEJ pathway. Although the difference between the HR-deficient and C-NHEJ-deficient lines was not significant, the C-NHEJ-deficient line showed higher mutation rate. It is suggested that when the C-NHEJ pathway is absent, DSBs are repaired not by the error-free HR pathway but by the error-prone A-NHEJ pathway, resulting in higher mutation rate.

In this study, it was suggested that DSBs induced in dry seeds of *A. thaliana* by heavy-ion beams are mainly but not entirely repaired by the C-NHEJ pathway. One possibility is that the embryo cells in the dry seeds are not uniform in the G1 phase. Another is that DSBs are not completely repaired in the G1 phase but in the following phase beyond the cell cycle check points.

*1 RIKEN Nishina Center

References
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