Dose-dependent mutagenic effects of 160-MeV/nucleon-argon beam in *Arabidopsis thaliana*

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Heavy-ion beams are used as an effective mutagens that induce localized mutations owing to their high linear energy transfer (LET). We previously reported a dose-dependent mutagenic effect of a 135-MeV/nucleon-carbon beam (30 keV/μm) in *Arabidopsis thaliana* in which the number of mutations per genome was dose-dependently increased in the range of 150–350.[1)] In this study, we investigated the dose-dependent mutagenic effect of a 160-MeV/nucleon-argon beam and its difference to that of a 135-MeV/nucleon beam.

Dry seeds of *Arabidopsis thaliana* (the Col-0 wild-type strain or CS16118 strain, which possesses a heterozygous null mutation in the *APG3* gene for measuring the mutation rate in M_1 generation[2)]) were irradiated with 160-MeV/nucleon-argon ions in doses of 0–120 Gy. The LET of the argon-ion beams was controlled to 188 keV/μm. Survival and mutation rates in M_1 generation were measured as previously described.[2)] Randomly selected ten M_2 plants were harvested from one self-pollinated M_1 plant. Genomic DNA was extracted from a mixture of the leaves of the ten M_2 plants. Five DNA pools were sequenced for each dose using HiSeq X-Ten sequencing systems (Illumina Inc.). The read sequences obtained were input into the mutational analysis pipeline, AMAP, as described previously,[3)] with some modifications. The mutation candidates were detected with GATK (HaplotypeCaller), PINDEL, and BREAKDANCER software. The number of mutations was counted as follows: For small mutations that included single nucleotide substitutions and <100 bp indels (insertions and deletions), each mutation was counted as one. For large mutations, that included ≥100 bp indels and chromosome rearrangements), the number of junctions in each mutant line were counted. Total number of these mutations per DNA pool was envisaged as the number of mutations per genome.

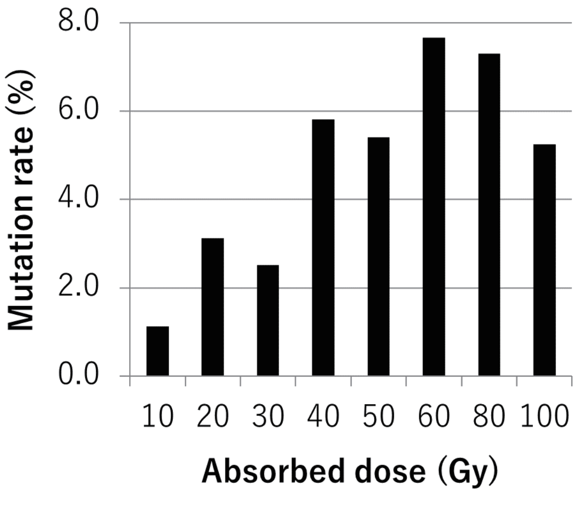

Fig. 1. Mutation rate in M_1 generation after irradiation of 160-MeV/nucleon-argon beam ($n \geq 500$).

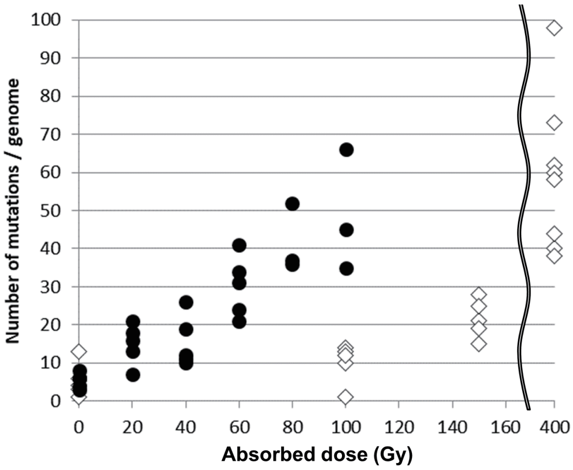

Fig. 2. Number of mutations in M_2 generation induced after irradiation of 188 keV/μm argon-ion beam (black circles) and 30 keV/μm carbon-ion beam (white rhombi).

In the M_1 generation, the average survival rate (flowering rate one month after cultivation[2)]) was 94% at 80 Gy-irradiation and decreased to 74% at 100 Gy-irradiation (data not shown). The mutation rate (appearance ratio of white sector mutation[2)]) indicated an increasing tendency with the increasing dose in the range of 10–80 Gy (Fig. 1). The highest mutation rate was 7.7% at 60 Gy-irradiation, which is as high as that previously reported (7.0% at 400 Gy with 30 keV/μm of LET; $p = 0.54$ by chi-square test).[2)] The decreased mutation rate at 100 Gy-irradiation may reflect the radiation overdose, as seen in the survival rate.

The number of mutations per genome in the M_2 generation after 188 keV/μm argon ion beam irradiation compared to that after 30 keV/μm carbon ion beam irradiation previously reported[1,4)] is shown in Fig. 2. After 400-Gy irradiation of the 30 keV/μm carbon-ion beam, the mutation rate in the M_1 generation was approximately 7%[2)] and the average number of mutations in the M_2 generation was 59 (Fig. 2). In contrast, after the 60-Gy irradiation of the 188 keV/μm argon-ion beam, the mutation rate in the M_1 generation was

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7.7% (Fig. 1) but the average number of mutations in the M_2 generation was 29 (Fig. 2). The difference in the average number of mutations in the M_2 generation under between the two irradiation conditions can be attributed to the content of the mutations. Among the number of mutations in the M_2 generation induced by the 30 keV/μm carbon-ion beam, only 4% were derived from large mutations, whereas 32% were obtained by 188 keV/μm argon-ion beam. Further analysis, for example, to compare the number of genes affected by the mutations including inversions that possibly cause the chromosomal position effect of the 30 keV/μm carbon-ion and the 188 keV/μm argon-ion beam may provide insight into the mutagenic characteristics of each beam.

References

1) Ishii *et al.*, RIKEN Accel. Prog. Rep. **52**, 216 (2019).
2) Y. Kazama *et al.*, Plant Biotechnol. **29**, 441 (2012).
3) K. Ishii *et al.*, Genes Genet. Syst. **91**, 229 (2016).
4) Y. Kazama *et al.*, Plant J. **92**, 1020 (2017).