Effect of LET on mutational function revealed by whole-genome resequencing of *Arabidopsis* mutants†

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Heavy-ion irradiation is a powerful mutagen that possesses high linear energy transfer (LET). Since the value of LET affects DNA lesion formation in several aspects, including the efficiency and density of double-stranded break along the particle path,[1,2] mutations induced after the DNA lesion repair would also be affected by the value of LET. Whole-genome resequencing is an effective way to assess the effect of the value of LET on mutation induction, and provides sufficient number of mutations from each mutant to perform statistical analyses.[3] Here, we investigated the differences in the mutation type induced by irradiation with two representative ions, namely C ions (LET: 30.0 keV/μm) and Ar ions (LET: 290 keV/μm), by whole-genome resequencing of the *Arabidopsis* mutants produced by these irradiations.

Dry seeds of *A. thaliana* were irradiated with C ions (30.0 keV/μm) or Ar ions (290 keV/μm) with doses found to induce 95% survival rates and the highest mutation frequencies.[4] For the C-ion and Ar-ion irradiations, doses of 50 and 400 Gy were adopted, respectively. Eight mutants showing morphological phenotypes were screened in the M_2 generation after each irradiation. Then, the phenotypes of the mutants were confirmed in the M_3 generation. In total, 16 mutants were selected, and DNA pools were extracted from 40 plants of their individual progeny. The extracted DNA was sequenced using the HiSeq 2500 and HiSeq 4000 sequencing systems (Illumina Inc., `https://www.illumina.com`). The obtained reads were input into AMAP, as described previously.[5]

The rearrangements including translocations and large deletions ($\geq$ 100 bp) that were induced by Ar ions were 4.6 times more frequent than those induced by the C ions; the average number of rearrangements in a mutant genome was 10.3 and 2.3 for Ar ions and C ions, respectively (Fig. 1). These differences were statistically significant ($P < 0.01$; two-sided Welch's t-test). Both Ar and C ions induce rearrangements. However, more complicated rearrangements occurred following Ar-ion irradiation, in which fragments of several hundred kbp to several Mbp were produced and joined with direction or positions different from those of the original ones. Conversely, Ar ions induced small mutations including base substitutions and small indels (< 100 bp), which were 2.3-fold less frequent than C ions: the average number of small mutations in a mutant genome was 18.3 and 41.6 for Ar ions and C ions, respectively. This difference was also statistically significant ($P < 0.01$; two-sided Student's t-test).

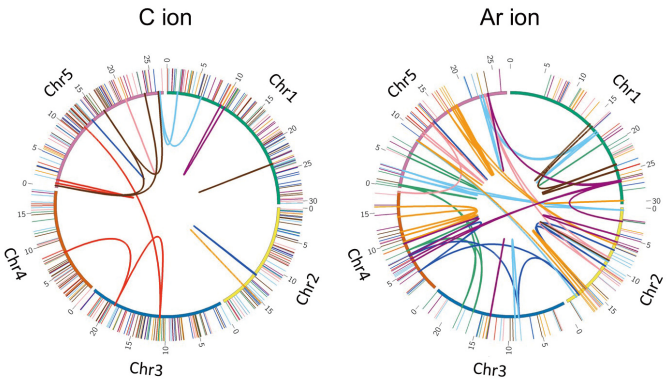

Fig. 1. Mutations in each of the eight mutants induced by C- and Ar-ion irradiation. The rearrangements are plotted as lines on the interior of the circles. Small mutations are indicated by lines on the exterior of the circles. The mutations in each mutant are differently colored.

Table 1. Sum of homozygously mutated genes detected in eight mutants.

	Amino acid changes	Truncation or loss of whole gene	Total
C ion	30	11	41
Ar ion	8	66	74

The effects on gene mutations were also different between C-ion and Ar-ion irradiations. The sum of homozygously mutated genes in eight individual mutants after irradiation with Ar and C ions are shown in Table 1. After C-ion irradiation, amino-acid changes were frequently observed, which were caused by small mutations. On the other hand, Ar-ion irradiation frequently induced truncations of genes or losses of whole genes caused by rearrangements.

These data demonstrate that the nature of mutations is significantly different between beams with different LET values. Such a selective irradiation will be a powerful tool for forward genetics as well as studies on chromosomal rearrangements in conjunction with the technics of mutation detection through high-throughput sequencing.

References
1) D. Alloni *et al.*, Radiat. Res. **173**, 263 (2010).
2) N. A. Franken *et al.*, Radiat Oncol. **6**, 64 (2011).
3) K. Shirasawa *et al.*, Plant Biotechnol. J. **14**, 51 (2016).
4) Y. Kazama *et al.*, Plant Biotechnol. **25**, 113 (2008).
5) K Ishii *et al.*, Genes Genet. Syst. **91**, 229 (2016).

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