

Whole genome sequencing analysis for detecting mutations induced by carbon- and argon-ion irradiations of rice (*Oryza sativa* L.)

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A mutation analysis with high-throughput sequencing is a powerful tool for characterizing the molecular nature of mutations induced by mutagen at the whole-genome level. We have investigated the mutation effect following heavy ion-beam irradiation of rice (*Oryza sativa* L. cv. Nipponbare), a monocotyledonous model plant, using high-throughput sequencing. In this study, we irradiated C-ion (LET: 30 keV/ μ m) or Ar-ion (LET: 290 keV/ μ m) to rice, and investigated numbers and types of mutations induced by both ions at whole-genome level to examine whether the difference in the LET value of the ions influences the numbers and sizes of mutations in rice genome.

We irradiated C-ion (175 Gy, LET: 30 keV/ μ m) or Ar-ion (10 Gy, LET: 290 keV/ μ m) to rice (*Oryza sativa* L. cv. Nipponbare) dry seeds. The plants from irradiated seeds (M_1 plants) were grown in a paddy field and self-pollinated to obtain M_2 seeds. Next year, we grew M_2 plants in a paddy field to collect mutant plants with visible phenotypes and harvest M_3 seeds from the each plant. For this study, we selected 11 and 4 mutant plants from C-ion and Ar-ion irradiations, respectively. Genomic DNA extraction for Whole-genome sequence was performed from the leaves of individual M_3 plants. Candidate mutation extraction were performed using “bioinformatics pipeline.”¹⁾ We extracted candidate mutations using a combination of the GATK, Pindel, Delly, and Manta programs. GATK adopted the following parameters of (-stand_call_conf 50 -A Coverage -A RMSMappingQuality -baq CALCULATE_AS_NECESSARY). Pindel was used following parameters of (-window_size 1 -report_long_insertions -report_breakpoints -minimum_support_for_event 3 -min_inversion_size 10). Delly and Manta were run with default parameters. All mutations detected were visually confirmed using Integrated Genomics Viewer (IGV).²⁾

We detected 1718 mutations from 11 C-ion-irradiated mutants, and 426 mutations from 4 Ar-ion-irradiated mutants. The average numbers of total mutations in a mutant genome were 156.2 and 106.5 for C-ion and Ar-ion irradiations, respectively. The most abundant mutations induced by both C-ion and Ar-ion irradiation were single-nucleotide variants (SNVs). The average numbers of SNVs in a mutant genome were 123.2 and 86.8 for C-ion and Ar-ion, respectively (Fig. 1). The second most abundant mutations induced by both C-ion and Ar-ion irradiation were small (<100 bp) insertions and deletions (indels). The average numbers of indels in a mutant genome were 31.5 and 16.3 for C-ion and Ar-ion, respectively. There were large deletions

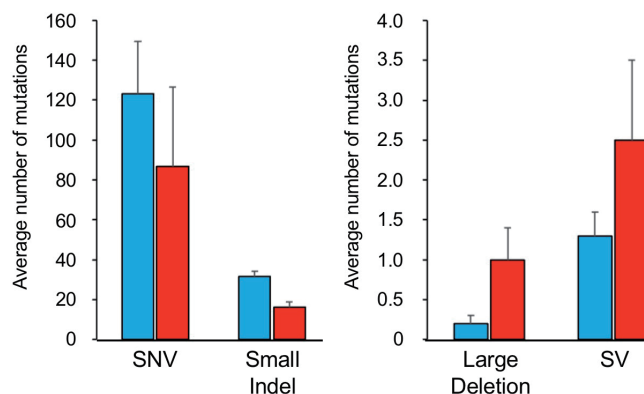


Fig. 1. Average number of SNV, small Indel, Large deletion, and SV induced by C-ion (blue) and Ar-ion (red) irradiations. Error bars indicate standard error.

(≥ 100 bp) and structural variations (SVs), such as inversion and translocation in a mutant genome derived from both C-ion and Ar-ion irradiations. The average numbers of large deletions and SVs in a mutant genome were 0.2 and 1.3 for C-ion, and 1.0 and 2.5 for Ar-ion, respectively. Comparing the mutations induced by C-ion and Ar-ion, the number of small mutation, such as SNVs and small indels, tended to be higher in the C-ion irradiation than in Ar-ion irradiation. Conversely, the number of both large deletion and SVs tended to be higher in Ar-ion than in C-ion. These tendencies corresponded to the results observed in the irradiation of Arabidopsis seeds.³⁾ They compared the number of mutations induced by C-ion (LET: 30 keV/ μ m) or Ar-ion (LET: 290 keV/ μ m) irradiation using Arabidopsis, a dicotyledonous model plant, at the whole-genome level, and demonstrated that Ar-ions induced rearrangements (including large-deletion and SVs) more frequently than C-ions.³⁾ They also indicated that Ar-ions induced SNVs less frequently than C-ions. The findings obtained in this study and that of Kazama *et al.* (2017) suggested that C-ion irradiation tends to induce small mutations predominantly, whereas Ar-ion irradiation tends to induce higher number of large deletions and SVs than C-ion irradiations of both rice and Arabidopsis genomes. Because the sample size was not sufficient to compare the numbers of each mutation induced by C-ion and Ar-ion statistically in this study, we plan to increase the sample size to perform a more detailed analysis.

References

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- 2) J. T. Robinson *et al.*, Nat. Biotechnol. **29**, 24 (2011).
- 3) Y. Kazama *et al.*, Plant J. **92**, 1020 (2017).

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