Estimation of efficient dose for heavy-ion beam mutagenesis by whole-genome mutational analysis in *Arabidopsis thaliana*

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Heavy-ion beams are used as an effective mutagen that induces localized mutations due to its higher linear energy transfer (LET). The observation of the occurrence ratio of albino mutants as the mutation frequency in the M2 generation after carbon-ion beam irradiation (30 keV/μm) at doses of 50–500 Gy showed that the mutation frequency increased with increasing dose between 50–350 Gy and plateaued at 400 Gy.[1)] However, the measurement of mutation frequency by visual screening of mutants from a large number of irradiated plants is time-consuming. In this study, we tried to estimate an efficient dose for heavy-ion beam mutagenesis by performing whole-genome mutational analysis on relatively small numbers of irradiated plants.

Dry seeds of *Arabidopsis thaliana* (the Col-0 strain) were irradiated with $^{12}C^{6+}$ (135 MeV/nucleon) ions, at doses of 0–350 Gy. The LET of C ion beams was controlled to 30 keV/μm. M_2 seeds were harvested from self-pollinated M_1 plants, and ten M_3 plants were harvested from one self-pollinated M_2 plant. Genomic DNA was extracted from the mixture of leaves of the ten M_3 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, with some modifications.[2)] In short, after mapping the read sequences to the reference genome sequence (TAIR10) with BWA (BWA-MEM) software, the mutation candidates were detected with GATK (HaplotypeCaller), PINDEL, and BREAKDANCER software. Then, the AMAP filtered out the false-positives by using its own algorithm.

In spite of the process of filtering out false-positives, confirmation of the mutation candidates in one DNA pool (350-Gy irradiation) by using Integrative Genomics Viewer (IGV) software revealed that 81% of the candidates were still false-positives (data not shown). We configured additional criteria for the determination of false-positives. Among the mutation candidates output from GATK, those with the 'QUAL' value (calculated by GATK) less than 150 or 'MAPPING_QUAL' value (calculated by BWA) less than 60 were treated as false-positives. Among the mutation candidates output from PINDEL, those with the ratio of the number of read sequences supporting the mutation to that of mapped read sequences less than 0.2 or with the number of reads supporting the mutation less than 5 were treated as false-positives. Among the mutation candidates output from BREAKDANCER, those with the ratio of the number of read sequences supporting the mutation to that of mapped read sequences less than 0.15 were treated as false-positives. Also, among the mutation candidates output from PINDEL or BREAKDANCER, two candidates from different DNA pools overlapping one another by more than 80% of their regions were treated as false-positives. By using IGV software, it was revealed that 21% of the candidates in the DNA pool (350-Gy) were false-positives after these additional filtrations and that 9% of the positive mutations were filtered out by the additional filtrations. The mutation candidates in all DNA pools were tested with the additional filtrations and checked using IGV software.

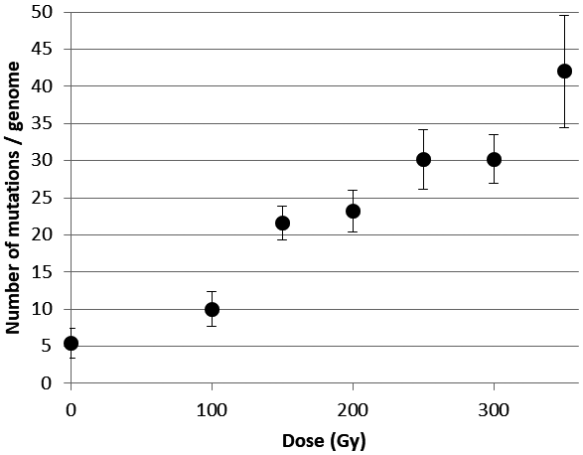

Fig. 1. Average number of mutations. Five DNA pools were analyzed at each dose.

The number of mutations per genome increased monotonically as the irradiation dose increased and did not plateau until 350 Gy (Fig. 1). In this study, it was suggested that irradiation at 350 Gy is efficient in the range of 0–350 Gy. Although the means for evaluating the mutation frequency in this study was different from that in the previous study,[1)] the tendency of increase in the mutation frequency in proportion to the irradiation dose was similar. The mutation analysis method in this study covered all mutations in the whole genome while the mutation analysis by visual screening covered only those in genes related to the development of chloroplasts. Because this method can determine the type (single nucleotide substitution, deletion, insertion, inversion, or chromosomal rearrangement) and the size of each mutation, the previous study revealed the LET-dependent effect for mutation induction: heavy-ion beams with higher LET can induce chromosomal rearrangements or large deletions more frequently than those with lower LET.[3)] In this study, it was also revealed that this method can be applied to the dose-dependent effect for mutation induction.

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References
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