Characteristics of genomic rearrangements induced by heavy-ion beam irradiation in *Arabidopsis thaliana* †

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Among plants, the molecular nature of mutations induced by heavy-ion beam irradiation has been mainly characterized in *Arabidopsis thaliana*. We previously reported that an Ar-ion or Fe-ion beam with a high LET (≥ 290 keV/μm) often induces large deletion mutations and genomic rearrangements.[1), 2)] However, such genomic rearrangements could not be fully characterized by the conventional method using polymerase chain reaction and sequencing analysis on restricted chromosomal regions or loci. Whole-genome resequencing might help fully detect the rearrangements. In the present study, we characterized the total mutations in the mutant genomes by whole-genome resequencing, and the mutagenic effects of the high-LET heavy-ion beams were evaluated.

Resequencing of three mutant lines of *A. thaliana* ecotype Col-0 was performed at the Takara Dragon Genomics Center (Takara Bio Inc., Mie, Japan) using one lane of the HiSeq 2000 sequencing system (Illumina Inc., CA, USA). Genomic DNAs for the resequencing were isolated from 40 plants of each mutant line (M_3 generation) derived from Ar-ion beam irradiation (290 keV/μm, 50 Gy). The reads obtained from the resequencing were mapped to a reference genome (TAIR10). The total mutations in the mutant genomes were detected by using SAMtools (v0.1.16),[3)] Pindel (v0.2.4.d),[4)] and BreakDancer (v1.1)[5)] algorithms, and the mutations observed at least in two mutants were excluded, being considered as background mutations harbored in ecotype Col-0 in our laboratory.

The three mutant lines selected for the resequencing were prescreened by array-comparative genomic hybridization (CGH), wherein the induced mutations were partially identified. The mutant lines Ar-57-al1, Ar-365-as1, and Ar-443-as1 showed no large deletions (≥ 200 bp), deletions between several hundred bp to several kbp with genomic rearrangements, and a 600-kbp deletion with a genomic rearrangement, respectively. As a result of resequencing followed by mutation detection, total mutations including base substitutions, duplications, in/dels, inversions, and translocations were detected. In mutant line Ar-57-al1, the size range of the detected deletions was from 1 to 75 bp, and these results were in agreement with those from array-CGH. Large deletions and genomic rearrangements were detected by Pindel and BreakDancer, and all of the deleted regions flagged by array-CGH were detected by the resequencing-based method in Ar-365-as1 and Ar-443-as1.

The numbers of homozygous-mutated genes, in which mutations would be expected to affect the mutant phenotypes, were calculated as seven, five, and eleven in the Ar-57-al1, Ar-365-as1, and Ar-443-as1 mutant lines, respectively. The candidate genes responsible for the mutant phenotypes we focused on were found from the homozygous-mutated genes in each mutant line. These results indicate that the mutation detection platform using resequencing data has the appropriate performance standards to detect total mutations induced by radiation treatment.

Resequencing revealed that the three mutants harbored complex genomic rearrangements. In the rearrangements of Ar-365-as1, for example, two inverted fragments were detected in chromosome 2, and one of the fragments translocated in the same chromosome (Fig. 1). In total, 22 DNA fragments were found to have contributed to the complex genomic rearrangements. Of these, 19 fragments were involved in the intra-chromosomal rearrangements. Accelerated Ar ions with high LET lead to dense ionization along the particle path. Therefore, it is suggested that DNA damage induces juxtapositions in the mutant genomes, and that clustered DNA fragments, contributing to the intrachromosomal rearrangements and large deletions, are generated.

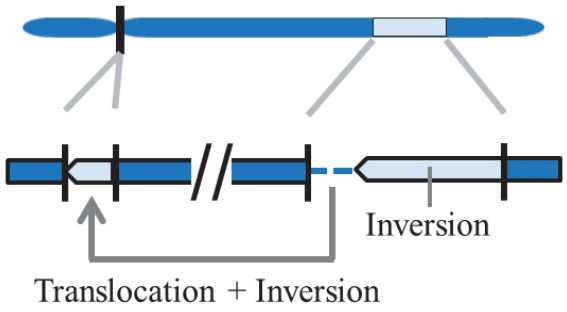

Fig. 1 Schematic representation of genomic rearrangements induced by Ar-ion beam irradiation. The rearrangements were detected in chromosome 2 of mutant line Ar-365-as1.

References
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