Responsible gene analysis of phenotypic mutants revealed the linear energy transfer (LET)-dependent mutation spectrum in rice[†]

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Linear energy transfer (LET), which refers to the amount of energy deposited per unit length of a particle's path (keV/μm), is an important parameter for ion beam mutagenesis. We previously demonstrated that the C ion irradiation (30 keV/μm) of Arabidopsis results in single nucleotide variants (SNVs), small indels (<100 bp), and translocations in the responsible genes for mutant phenotypes, with SNVs and small indels representing more than 80% of the mutations.[1)] Although the mutation frequencies induced by C ions (290 keV/μm) and Ar ions (290 keV/μm) are relatively low, both irradiations frequently result in large deletions ($\geqq$100 bp) in the responsible genes for mutant phenotypes.[2)] During an earlier whole-genome sequencing (WGS) analysis of Arabidopsis mutant lines conducted to compare the effects of Ar and C ions, the Ar ion (290 keV/μm) caused chromosomal rearrangements and large deletions more frequently than the C ions (30 keV/μm), whereas the C ions induced more SNVs and small indels in the genomes.[3)] These results indicated that the LETs of ion beams clearly affected DNA mutations in Arabidopsis. However, the effects of LETs of ion beams for mutation induction in plants other than Arabidopsis are largely unknown.

In this study, we irradiated rice seeds (dry or imbibed seeds) with C, Ne, or Ar ions with various LETs. The resulting mutant plants were isolated and the mutations in the responsible genes for the mutant phenotypes were examined using Sanger sequencing, whole-exome sequencing, or whole-genome sequencing to determine whether the mutation types differed among the LETs. We classified the mutations into the following four types: SNVs, small deletions (<100 bp), large deletions ($\geq$100 bp), and chromosomal rearrangements (CRs; inversions or translocations). We complied the mutations according to the ion beam LETs and compared the tendencies of each irradiation. For the C ion (23, 30, 50, or 57 keV/μm) and Ne ion (63 or 70 keV/μm) irradiations, small deletions were the most frequent DNA mutations (Fig. 1). In contrast, the most frequent mutations induced by the Ar ion (290 keV/μm) irradiation were large deletions. Thus, we compared the proportions of the four types of mutations between the

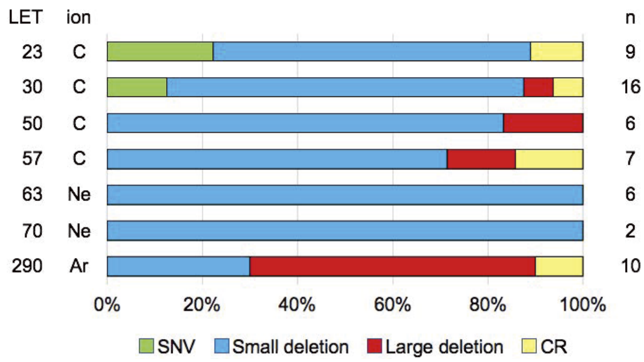

Fig. 1. Mutations on responsible genes classified on the basis of the LET.

Ar ion and C ion irradiations, which revealed significant differences ($p < 0.01$; Fisher's exact test). The differences were also significant when we compared the proportions of the mutations between the Ar and Ne ion irradiations ($p < 0.01$; Fisher's exact test). However, the differences in the proportions of the mutations between the C and Ne ion irradiations were not significant ($p > 0.05$; Fisher's exact test). Our data indicated ion beams with LETs of 23, 30, 50, 57, 63, and 70 keV/μm tended to cause small deletions, whereas the Ar ion irradiation tended to induce large deletions. These observations were consistent with the results of our previous studies on Arabidopsis mutants irradiated with C ions (23 or 30 keV/μm[1)]) or Ar ions (290 keV/μm[2,3)]). Thus, C ions (23 or 30 keV/μm) tend to induce small deletions, whereas Ar ions (290 keV/μm) tend to induce large deletions in the responsible genes for mutant phenotypes in rice and Arabidopsis. Therefore, we inferred that in rice and Arabidopsis, C ion (23 or 30 keV/μm) irradiations are suitable for efficiently inducing null mutants, whereas an Ar ion (290 keV/μm) irradiation is suitable for producing mutants with large deletions that can disrupt tandemly arrayed genes. Future investigations should clarify whether plant species other than rice and Arabidopsis exhibit similar tendencies regarding genetic mutations induced by ion beam irradiations. If these tendencies are common among plant species, ion beams with specific LETs may be used to induce particular mutation types, with important implications for research and plant breeding.

References

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